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PROCEEDINGS

OF THE

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EXPERIMENTAL BIOLOGY AND MEDICINE

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SCIENTIFIC PROCEEDINGS

One hundred ninth meeting.

ABSTRACTS OF COMMUNICATIONS.

Cornell University Medical College, October 17, 1920.
President Calkins in the chair.

1 (1583)

The oxidation of sulfur by microörganisms.

By **SELMAN A. WAKSMAN** and **J. S. JOFFE.**

[*From the New Jersey Agricultural Experiment Station, New Brunswick, N. J.*]

Two groups of phenomena are to be considered in the study of the sulfur cycle: (1) the reduction phenomena and (2) the oxidation phenomena. The first result in the production of hydrogen sulfide and the second in the oxidation of H_2S to S and of the latter to H_2SO_4. Both groups of phenomena result from activities of microörganisms. Winogradsky was the first investigator to have definitely demonstrated the rôle of microbes in the oxidation of the H_2S to S, and of the latter to H_2SO_4, but it is characteristic that the organisms studied by Winogradsky (*Beggiatoa* and *Thiothrix*), never turned the medium acid: this was explained by the presence of sufficient $CaCO_3$ in the culture to neutralize any acid formed. Keil isolated the two organisms in pure culture only about thirty years after the work of Winogradsky was carried out.

The sulfur oxidizing bacteria were usually divided into four groups, namely (1) Thread-forming colorless bacteria, accumulating sulfur within their cells. The *Beggiatoa* and *Thiothrix* are representative of this group. (2) Non-thread forming, colorless bacteria, accumulating sulfur within their cells. Here are referred forms (*Thioploca*, *Thiovulum* etc.) of various sizes and shapes, the distinguishing differences being the facts that they oxidize H_2S, accumulate sulfur within their cells, are colorless and non-thread forming. Some of these have been isolated in pure culture. (3) Purple bacteria. Some of these seem to play a part in the sulfur cycle, although none of the sulfur forms have yet been isolated in pure culture. (4) Colorless, non-thread forming sulfur oxidizing bacteria which do not accumulate sulfur within their cells, but which produce an abundance of sulfur (from H_2S and thiosulfates) outside of their cells. The two characteristic and most important forms belonging to this group are the *Thiobacillus denitrificans*, anaerobic, deriving its oxygen from the decomposition of nitrates; and *Thiobacillus thioparus*, which oxidizes thiosulfates, H_2S, and S, allows an extensive accumulation of sulfur from the first two and allows the medium to become distinctly acid. The work on this group of organisms has been carried on chiefly by Beijerinck and associates.

It is suggested here to add another group of sulfur-oxidizing bacteria, which was isolated and is being extensively studied at the New Jersey Agricultural Experiment Station. This group (5) will comprise bacteria similar to group 4 in their morphology, although much smaller in size (less than 1 μ. in length) and distinctly different physiologically. They do not act upon thiosulfates and H_2S, only upon elementary sulfur and allow the medium to become acid up to a P_H of 0.8–1.2.

These organisms have been isolated from composts consisting of sulfur, phosphate rock and soil, where the sulfur oxidation is very strong; they were isolated by means of a purely inorganic culture medium, consisting of minerals with elementary sulfur as the only source of energy. These organisms are autotrophic and do not need any organic substances for their development, the carbon being derived from the CO_2 of the air. The sulfur is oxidized very rapidly with the production of sulfuric acid. When the medium is poor in neutralizing substances as well as in inorganic buffering substances the accumulation of acids will soon reach such a concentration that the growth of the organisms may be stopped; in the presence of the proper neutralizing agents, such as tricalcium phosphate, sodium bicarbonate, or in the presence of sufficient amount of buffering agents, such as di-basic-phosphates and bicarbonates, the acid produced interacts with the basic element giving salts or acid salts tending to make the medium less acid. But as soon as the neutralizing agent is used up, the acid begins to accumulate, if there is an excess of elementary sulfur in the medium. It is characteristic to note here one thing: while the lack of acid production in the work of Winogradsky was explained by the fact that the presence of sufficient carbonates in the medium kept it at a neutral point, we find, in the case of our organism, that the neutralization of the basic substances is accomplished at a P_H 3.6 to 2.0, which is a distinctly acid zone, in fact even a more acid zone than the final acidity of the majority of acid producing bacteria (lactic, acetic, etc.) and yeasts.

We reported elsewhere that the sulfur-oxidizing bacteria, which we have isolated and a complete description of which will be published soon, produce a greater concentration of acids and remain alive in that acid medium, than any biological phenomena ever known.

Age of Culture.	P_H.	Titrat. Acidity c.c. N/10 Alkali per 5 c.c. of Culture.	Mgs. in 100 c.c. of Solution.		Sulfur Dissolved Mgs.	Soluble + Phosphates (as P) Mgs in 100 c.c.
			Sol. SO_4	Insol. SO_4		
At start..........	5.4	0.8	209	0	0	45
2 days..........	5.3	0.9	211	0	—	47
4 "	4.0	1.3	240	40	20	60
8 "	2.7	3.9	444	56	87	87
12 "	2.3	5.0	253	378	140	183
23 "	2.4	5.4	300	275[1]	115	194
	2.1	6.2	314	310	158	196
30 "	1.6	9.6	530	360	210	194

What we attempted in our work is not only the isolation of strong sulfur-oxidizing organisms, but the production of acid at such a rate and under such conditions that it should transform insoluble tri-calcium phosphate into soluble phosphates and phosphoric acid. The insoluble phosphates are used as neutralizing agents.

---

[1] The decrease is due to the fact that the determinations were based on separate flasks, in which the ratio between the soluble and insoluble $SO_4^=$ was not alike, for some reason or another.

2 (1584)

Formulæ for the determination of the correlations of size and of growth increments in the developing organism.

By **J. Arthur Harris.**

[*From the Station for Experimental Evolution, Cold Spring Harbor, L. I.*]

In the analysis of the growth of the higher organism it is essential to obtain definite measures of the interrelationship between certain measured magnitudes. Those which require consideration are the following:

(1) The correlations between the actual size of the organism at the various stages[1] of growth. (2) The correlations between growth increments of the organism during the several growth periods. (3) The correlations between the size of the organism at any stage and any or all subsequent growth increments.

The labor of determining these correlations by ordinary methods is excessive. If the first set of correlations (1) be determined by taking all moments about 0 as origin,[2] we may solve problems (2)–(3) as follows.

Problem 2.—To determine the correlations between growth increments from the moments and product moments of size at the several growth stages.

Let w, x, y, z be the dimensions of the organism at growth stages p, q, r, s. The growth increment during the intervals $q-p$, $r-q$, $s-r$ will then be $i_{pq} = x-w$, $i_{qr} = y-x$, $i_{rs} = z-y$.

The moments $\Sigma(x)$, $\Sigma(x^2)$, $\Sigma(y)$, $\Sigma(y^2), \cdots$, and the product moments $\Sigma(wx)$, $\Sigma(wy), \cdots$, $\Sigma(yz)$ are available for the correlations between size, which are required on their own account (Problem 1).

The constants for growth increments are given by well-known formulæ

---

[1] Growth stage denotes any given moment of time at which series of organisms of the same age are measured. During development it is, therefore, synonymous with age. The absolute size of the organism or any of its parts at a given growth stage is the only character of the organism available for consideration.

Growth period denotes the period of time elapsing between the sth and the $s+n$th growth stage, where s is any growth stage. Growth increment denotes the increase in size during any such period.

[2] Harris, J. Arthur, *Amer. Nat.*, 1910, xliv, 693–699.

$$\bar{i}_{pq} = \bar{x}\text{-}\bar{w}, \text{ etc.},$$
$$\sigma^2_{i_{pq}} = [(\Sigma(w^2) + \Sigma(x^2) - 2\Sigma(wx))]/N - \bar{i}^2_{pq},$$

and similarly for $\sigma_{i_{qr}}, \sigma_{i_{rs}} \cdots$.

The product moment for any two growth increments, say i_{pq} and i_{rs}, is

$$\Sigma(i_{pq}i_{rs}) = \Sigma(wy) - \Sigma(wz) - \Sigma(xy) + \Sigma(xz).$$

In the special case in which three *consecutive* stages, say w, x, y, are involved we write

$$\Sigma(i_{pq}i_{qr}) = \Sigma(wx) - \Sigma(wy) + \Sigma(xy) - \Sigma(x^2).$$

Problem 3.—To determine the correlation between the size of the organism at any stage and any growth increment.

The notation of problem (2) may be used. The physical constants for the growth stages and growth increments have been given. The product moments are

$$\Sigma(wi_{pq}) = \Sigma(wx) - \Sigma(w^2),\ \Sigma(xi_{qr}) = \Sigma(xy) - \Sigma(x^2),$$
$$\cdots,\ \Sigma(wi_{qr}) = \Sigma(wy) - \Sigma(wx),\ \ \Sigma(wi_{rs}) = \Sigma(wz) - \Sigma(wy),\ \text{ etc.}$$

Illustrations of applicability will be given elsewhere.

3 (1585)

The carbon dioxid dissociation curve and the arterial and venous carbon dioxid tension of human blood in health and in disease.

By **JOHN P. PETERS, JR.** and **DAVID P. BARR.**

[*From the Russell Sage Institute of Pathology and the Second Medical Division of Bellevue Hospital, New York, N. Y.*]

A method for the direct determination of the carbon dioxid tension of human arterial and venous blood has been applied to a series of normal and pathological subjects. The method is similar to one recently described by Means, Bock and Woodwell[1] for the determination of arterial carbon dioxid tension, but was developed and applied by us independently before the appearance of Means' paper. It is a development of the work of Henderson and Haggard[2] on the "Hemato-Respiratory Function" and consists in the simultaneous determination of the carbon dioxid dissociation curve of the blood at body temperature and the carbon dioxid content of both the arterial and venous blood as they occur in the body.

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[1] Means, Bock and Woodwell, *Transactions Am. Assn. Physicians*, 1920.

[2] Henderson and Haggard, *Journ. Biol. Chem.*, 1919, xxxix, 163.

This method has been applied in whole or in part to twenty-three subjects, on whom thirty-eight observations have been made. The group studied consisted of three normal persons, seven patients with decompensated cardiac disease, six patients with severe anemia, four with diabetes, two with emphysema, one with polycythemia and one with chronic nephritis. Whenever it was found practicable the alveolar carbon dioxid tension was also determined by the Haldane[1] method.

The normal limits of variation in height of the carbon dioxid dissociation curve at 37.5° C. were found to agree with those established by previous observers. At 42 mm. CO_2-tension the limits of variation of the carbon dioxid combining power of whole blood are 43 and 56 volumes per cent. The height of the normal resting dissociation curve is as Christiansen, Douglas and Haldane [2] previously found, characteristic for each individual.

In three of the seven patients with cardiac decompensation the dissociation curve lay below the normal limits, indicating a real reduction of the available alkali of the blood. The carbon dioxid capacity of the venous plasma was, however, normal in these cases. The difference between the CO_2-capacity of whole blood and that of venous plasma found in these cases may be explained by the fact that the carbon dioxid capacity of the venous plasma as determined by the Van Slyke[3] method is dependent upon the carbon dioxid content of the blood as it exists in the veins. Therefore, in conditions like cardiac decompensation, in which there is a retention of carbon dioxid in the venous blood, the carbon dioxid combining capacity of the venous plasma is inapplicable as a measure of the available alkali of the blood. For this reason whole blood should be used or else the whole blood should be equilibrated with a standard CO_2-air mixture before the plasma is removed.

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[1] Haldane and Priestley, *Journ. Physiol.*, 1904–5, xxxii, 225.

[2] Christiansen, Douglas and Haldade, *Journ. Physiol.*, 1914, xliii, 244.

[3] Van Slyke, *Journ. Biol. Chem.*, 1917, xxx, 347.

The dissociation curve in severe anemia is more nearly horizontal and lies at a higher level than does the normal dissociation curve at carbon dioxid tensions that exist in the body. This is due to the diminution of the hemoglobin, which has the power, according to Parsons[1] and Henderson[2], of combining with a part of the alkali of the blood. The diminution of hemoglobin renders a larger proportion of the alkali of the blood available for combination with carbon dioxid at low tensions of CO_2. This reduces the "buffer" action of the blood against carbon dioxid so that for a given change of CO_2-tension the corresponding change in hydrogen-ion concentration is greater than normal. This is offered as a partial explanation of the exertional dyspnea found in these cases.

The P_H of blood exposed to a carbon dioxid-air mixture of the same CO_2-tension as that existing in the alveolar air obtained by the Haldane method was calculated from the $H_2CO_3/BHCO_3$ ratio and found to vary between 7.42 and 7.29, with an average of 7.35. In general the alveolar carbon dioxid, dissociation curve and alveolar P_H during rest vary in different individuals but are constant and characteristic for any given individual.

The arterial CO_2-tension, as determined in three normals, is also a characteristic of the individual, as is the arterial P_H. The limits of variation of the arterial P_H are, however, greater than those of the alveolar P_H, being 7.23 to 7.45. As a natural corollary to this it is found that, contrary to general opinion, the alveolar tension is not always the same as that of the arterial blood. The arterial tension may be as much as 10 or 11 mm. Hg. higher than the alveolar even in normal resting persons.

An empirical equation to correct the carbon dioxid tension of the arterial and venous blood for oxygen-unsaturation is proposed. It assumes that the action of oxygen on the carbon dioxid combining capacity of whole blood is a function of the concentration of hemoglobin in the blood and the ratio of reduced to oxy-hemoglobin. The equation employed was:

$$\frac{6.38}{18.5}\times\left(\begin{matrix}\text{O}_2\text{ capacity of blood}\\ \text{in vol. per cent.}\end{matrix}\right)\times\frac{\left(\begin{matrix}\text{Per cent. O}_2\text{ unsaturation}\\ \text{of blood}\end{matrix}\right)}{100} =$$

---

[1] Parsons, *Journ. Physiol.*, 1917, li, 440, 1920, liii, 340.

[2] Henderson, L. J., *Journ. Biol. Chem.*, 1920, li, 401.

D vol. per cent. (Amount by which the carbon-dioxido combining capacity of blood is increased as a result of the effect oxygen unsaturation).

As applied to the figures given by Haldane[1] and by Joffe[2] it shows an error of only 10 per cent. between CO_2-tensions of 30 and 70 mm. inclusive. This equation has been employed in the calculation of arterial and venous carbon dioxid tension. Although the differences in CO_2 tension between arterial and venous blood are greater and more variable than the results of indirect methods have led us to believe, the slope of the dissociation curve and the effect of oxygen combined prevent any change in the hydrogen-ion concentration. The P_H of venous blood as calculated from the $H_2CO_3/BHCO_3$ ratio is practically the same as that of arterial blood.

In cardiac dyspnea the difference between alveolar and arterial CO_2-tension was always much greater than normal, varying from 13 to 19 mm. Hg. This indicates some impairment of the mechanism for the elimination of carbon dioxid in the lungs: either that parts of the lungs are unaerated or that they are imperfectly ventilated. The arterial P_H was once absolutely diminished and once relatively diminished, indicating the presence of a true carbon dioxid retention. Most of the patients studied did not recover compensation, but in one case the carbon dioxid retention disappeared completely and the arterial P_H and the relation of arterial to alveolar carbon dioxid tension, returned to normal. The difference between arterial and venous carbon dioxid content and arterial and venous carbon dioxid tension was only occasionally greater than normal. A retarded circulation rate is therefore not a necessary concomitant of cardiac decompensation with dyspnea. The venous and arterial P_H were practically identical even in those cases with an increased difference between arterial and venous carbon dioxid tension because of the greater oxygen-unsaturation of the venous blood.

In severe anemia not only is the dissociation curve higher and more nearly horizontal than normal at CO_2-tensions that exist in the body, but the compensating effect of oxygen is greatly diminished because of the diminution of hemoglobin. In spite of the fact that the difference in carbon dioxid content and tension between arterial and venous blood is comparatively small, there is a very definite difference in P_H. The arterial P_H lies well to the alkaline side of the 7.35 line in those cases where there is a difference, while the venous point lies practically on the 7.35 line.

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[1] Christiansen, Douglas and Haldane, *Journ. Physiol.*, 1914, xliii, 244.

[2] Joffe, Poulton, Poulton and Poulton, *Journ. Physiol.*, 1920, liv, 129.

It is suggested tentatively as an explanation of this phenomenon, that the tissue CO_2-tension and P_H must lie at or above that of the venous blood and not in equilibrium with the arterial blood. As it is presumably the tissue CO_2-tension or hydrogen-ion concentration in the respiratory center which controls the respiratory mechanism, the tendency of the repsirations will be to maintain this constant rather than the hydrogen-ion concentration of the arterial blood. In normal persons arterial and venous P_H are practically identical because of the slope of the dissociation curve and the effect of oxygen. In anemia the effect of these compensating reactions is diminished so that true relations become more evident. It has already been demonstrated by Michaelis,[1] and others that the hydrogen-ion concentration of the venous blood is maintained constant at $P_H = 7.35$ with a variation of ± 0.08, which agrees well with our values for both arterial and venous P_H.

4 (1586)

Precipitin response in the blood of rabbits, following subarachnoid injections of horse serum.

By H. L. ALEXANDER.

[*From the Second Medical Division of Bellevue Hospital, and the Department of Medicine, Cornell University Medical College.*]

During the treatment of cases of cerebrospinal meningitis with antimeningococcic serum in a large Army hospital,[2] a curious reaction was repeatedly observed. This appeared in patients who, after having received several intraspinous treatments with serum, were given serum intravenously. While such injection was made into the blood stream, or immediately thereafter, some of the following signs and symptoms frequently appeared: flushing, sudden feeling of warmth, restlessness,—then, pallor, dyspnea, cyanosis, vomiting, and prostration. Epinephrin and atropin hyperdermatically, induced relief. These manifestations, apparently anaphylactic, occurred only after several days of intraspinous treatments had elapsed before initial intravenous serum therapy, but had no relation to the time of the last intraspinous injection. They were not noted when combined intravenous and intraspinous therapy was applied from the outset. Similar observations are described by Stone and Truit[1] in their report of a large series of cases of meningitis at Camp Funston, and Haden[2] confirms them in one of his case reports of meningitis.

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[1] Michaelis, Wasserstoffionenkonzentration, Berlin, 1914.

[2] Hospital of the American Embarkation Center, LeMans, France.

With this experience in mind, horse serum was injected into rabbits intraspinously, and the resulting precipitin formation in the blood was compared with that induced by similar intravenous injections. In a few instances, anaphylactins were studied.

Normal horse serum, without preservative, was used throughout these experiments. This was injected into the subarachnoid space of rabbits by introducing a No. 24 Luer needle attached to a glass syringe, through a sterile field just below the occipital ridge in the mid-line. The needle was carried forward and slightly downward until it punctured the occipito-atlantoid ligament. A yield of from 0.5 c.c. to 1.0 c.c. of spinal fluid was thus readily obtained. Leaving the needle in place, and disconnecting the syringe, a second syringe with a correct amount of serum was then attached. By slightly withdrawing the plunger, freedom from chance puncture of a vessel was assured, and the serum then slowly injected. No anesthetic was needed, the rabbit being securely tied in the prone position to a board and the head flexed and pulled forward by the ears.

Precipitin tests were made by mixing 0.3 c.c. of rabbit serum with 0.3 c.c. of normal horse serum. After incubation at 37° C. for one hour in a water bath, the tubes were placed in the ice chest overnight, and readings made the following morning. As controls, normal rabbit serum and normal sheep serum were used. In all instances, rabbits receiving subarachnoid injections were paralleled by rabbits injected with identical amounts of the same serum intravenously, and the bleedings and precipitin tests of each were done at the same time.

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[1] Stone, W. J. and Truitt, R. C. P., *Arch. Int. Med.*, 1919, xxiii, 282.

[2] Haden, R. L., *Arch. Int. Med.*, 1919, xxiv, 514.

Summary: (1) Rabbits receiving a single dose (0.5 c.c.) of normal horse serum into the subarachnoid space, produce precipitins in the blood in greater abundance, of higher titer, and which persist longer than those in control rabbits receiving a similar injection intravenously.

(2) Repeated subarachnoid injections (0.5 c.c.) of normal horse serum in rabbits, induce precipitins in the blood early. These may appear in high titer as soon as one week after the initial injection, whereas in rabbits similarly treated intravenously, no precipitins were found at this time. They may appear a few days thereafter and reach a high titer.

(3) No anaphylactic manifestations occurred in rabbits treated repeatedly with subarachnoid injections of normal horse serum when the precipitin content of the blood was high.

(4) Anaphylactins, as determined by passive transfer of anaphylaxis, were demonstrated in sera with high precipitin content.

(5) These experiments may explain clinical manifestations of intolerance to horse serum, observed when an initial intravenous injection of antimeningococcic serum followed a series of intraspinous injections of such serum.

5 (1587)

Experimental gigantism produced by feeding pituitary gland.

By **EDUARD UHLENHUTH.**

[*From the Rockefeller Institute for Medical Research, N. Y. City.*]

Growth of the individual stops, when the size is reached which is specific for the species to which the individual belongs. The causes which lead to the cessation of growth are not fully known. In man it happens sometimes, that growth continues beyond the normal maximum size of the species; this condition is known as gigantism. Clinical evidence points to the conclusion that at least one form of gigantism is due to an overfunction of the hypophysis gland. Attempts, however, to produce gigantism by feeding hypophysis to animals have been unsuccessful.

The experiments to be discussed presently will show that in salamanders hypophysis feeding produces gigantism and that it is the anterior lobe alone which possesses the ability of maintaining growth after the normal size of the species is reached.

The effect of the hypophysis diet depends, however, on the developmental stage of the salamanders. Larvæ do not respond to the anterior lobe diet; the growth-promoting effect of the gland commences after metamorphosis has taken place.

Of the species *Ambystoma opacum*, quite a number of specimens have been kept and measured for several years in my laboratory. In a chart the growth during three years is shown for four specimens; after the first year the animals grow very little and, at certain periods, may show even a decrease in size. The largest animal of this species raised in my laboratory was 115 mm. long, the largest normal animal in my possession at present is 111.5 mm. long. I went through the collections of several museums; the largest specimen I found measured 117.7 mm.; of two breeding females caught recently, one measured 112 mm., the other one 106 mm.

Of four animals raised in the laboratory from eggs of the same female and kept on a normal diet two were started on the anterior lobe at an age of 62 weeks, about 12 months after metamorphosis and about 4 months after sex maturity had been reached, and two animals were kept as controls. The result is shown in the growth curves. In spite of the advanced age both hypophysis-fed animals started to grow at a rate that, under normal conditions, would be characteristic of an early period after metamorphosis, while the controls continued to grow at a low rate, although they were fed so excessively large quantities of normal food that both of them succumbed finally. The largest control animal measured 115 mm., when it died; would it have survived and increased steadily at the same rate—which, however, would not be expected from the curves of other normal animals—it would measure 118 mm. today, whereas the largest hypophysis-fed animal, which died a few days ago, measured 138 mm. The surviving anterior lobe-fed animal is passed around together with the largest normal animal kept at present in the laboratory. The hypophysis-fed animal is about 131 mm. long and two years old, the normal animal measures 111.5 mm. and is three years old. In the species *A. opacum* feeding of the anterior lobe resulted in gigantism.

Similar experiments were made in the species *A. tigrinum.* The normal growth curve of this species seems to resemble the growth curve of *A. opacum.* After the first year the rate of growth becomes very low as shown in the slides; the records of this species do not go, however, beyond 80 weeks and it is uncertain, when growth in *A. tigrinum* stops. The largest animal that I could find in the museum collections measured 208.7 mm. Larger specimens are found in the western states, but these are most likely derived from larvæ in which by abnormal processes of the inner secretory system growth was greatly stimulated already during the larvæ period, and which on account of this abnormal condition studied as yet very little, cannot be used for comparison with our eastern race. The largest normal animal raised in my laboratory measures 195 mm.

Every single individual of this species fed on the anterior lobe of the hypophysis not only outgrew all the controls and other normal animals raised in my laboratory, but also the largest animals which I found so far in museum collections. In one series of five specimens the two smaller individuals were selected for the purpose of the experiment and started on anterior lobe at an age of 37 weeks, about 27 weeks after metamorphosis; the three larger specimens were kept as controls. The controls grew only little after this time and were soon outgrown by the experimental animals in which the high rate of growth that prevailed during the early period was not only maintained but even surpassed by the rate of growth produced by the hypophysis diet. The largest hypophysis-fed animal of this series measures now 264 mm. and still is growing most vigorously, while the largest control animal measures only 192 mm. Both animals are passed around. Similar results were obtained in the other series and at present I possess five hypophyseal giants of this species, coming from two different broods.

If the animals are fed on the posterior lobe of hypophysis growth is not only not stimulated, but even greatly retarded as may be seen from two live animals, a control and a posterior lobe fed animal, both descendants of the same female and of the same age.

Feeding of anterior lobe causes (1) a very marked acceleration of growth and (2) a continuation of growth beyond the specific size of the species resulting thus in hypophyseal gigantism. Feeding of posterior lobe has neither of these two effects, but even retards growth.

Since in these experiments the hypophysis was fed without the addition of normal food and in large doses, one may think that the results were caused not by the action of a specific substance contained in the hypophysis but merely by the greater food value of the gland. Part of the acceleration of growth may have been actually due to merely quantitative differences in the food substances; but it should be pointed out that it is impossible to renew growth by feeding even large quantities of normal food after growth has come practically to a standstill. As regards the continuation of growth beyond the normal size of the species, it is obvious that the alteration of this specific character of growth cannot be due to an increased amount of food and it seems, therefore, that at a stage where growth ceases or is greatly diminished under normal conditions, cell proliferation can be actually enforced by the specific growth-promoting substances contained in the anterior lobe of the hypophysis.

6 (1588)

Observations on bacterial metabolism.

By **J. HOWARD MUELLER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, New York City.*]

In the course of an investigation of the cultural requirements of certain of the pathogenic bacteria, a substance which occurs in meat infusion, and also in some of the proteins has been found to be essential for the growth of the streptococcus, and for certain strains of the pneumococcus. While it has thus far not been possible to isolate the compound in pure form, perhaps enough has been learned of its occurrence and properties to warrant a short note.

If an infusion of beef, or better, of beef heart muscle be prepared by boiling a pound of the chopped muscle in a liter of water, straining and filtering, and if 0.1 per cent. glucose and a nitrogen free inorganic salt mixture be added, it is found that the broth thus prepared is quite suitable for growth of the hemolytic streptococci. A P_H of 7.2–7.6 is most favorable, and no peptone or other nitrogenous material need be added. If, however, the meat infusion be mixed with 2 per cent. of wood charcoal, of the commercial brand called "Norit," and boiled for fifteen minutes and filtered, the streptococcus will no longer grow on the filtrate, after adding glucose, and salts, and adjusting the reaction. Evidently a substance has been quantitatively removed from the infusion by the charcoal which is required by the streptococcus for growth. The addition of 1 per cent. commercial peptone to such a charcoal treated infusion now renders it again suitable for growth, although the peptone itself, plus glucose and salts, will not give growth with the streptococcus.

Since the material which is removed by the charcoal is apparently present in commercial peptone, it seemed most probable that an amino-acid or polypeptide was in question. The addition of a sulphuric acid hydrolysate of casein to the charcoal treated infusion was next tried, and found to be quite as effective as the peptone. The hydrolysate is prepared by 18 hrs. boiling with 33 per cent. H_2SO_4, and the acid then removed with baryta. To rule out as far as possible the presence of non-protein impurities in the casein, a purified specimen was prepared by reprecipitating three times with acetic acid from Na_2CO_3 solution, and finally washing thoroughly with alcohol and ether. An acid hydrolysate of this casein proved equally active when added to the inactive infusion. Similarly, active preparations could be obtained by the use of a sulphuric hydrolysate of edestin and meat residue, and very weakly active preparations from egg white, but the hydrolysates of wheat gluten, gelatine, wool and silk were quite inactive. Published analyses of these proteins did not show any amino acid common to the ones furnishing active hydrolysates and lacking in the others. Acid hydrolysates of yeast and of salmon sperm were also inactive, showing that none of the constituents of nucleic acid were concerned.

A separation of the amino acids from a casein hydrolysate was therefore undertaken, first into groups by the butyl alcohol extraction method of Dakin. By this method, the monoamino acids are extracted almost quantitatively, and crystallize out of the alcohol as a yellow, granular material, easily filtered out and dried. The proline remains dissolved by the alcohol, while the hexone bases and the dibasic acids remain dissolved in the aqueous phase, unextracted by the alcohol. The active material passed over almost quantitatively with the monoamino acids.

Various methods of separating this mixture of monoamino acids have been tried. The only reagent so far obtained which precipitates the active material from the mixture of monoamino acids is mercuric sulphate in sulphuric acid solution. This reagent precipitates the following known amino acids: tryptophane, tyrosine, cystine and histidine. Pure preparations of all four of these amino acids have been tested with charcoal treated infusion and found negative.

For further separation of the compounds precipitated with mercuric sulphate, considerable quantities of casein had to be used, and the preliminary extraction of the monoamino fraction by Dakin's method was to be avoided if possible. It was found that mercuric sulphate precipitated the active material from the original hydrolysate, and further, that it was not even necessary to remove the sulphuric acid with baryta, but that one could neutralize the excess acid with crude sodium hydroxide. After filtering off the melanin thus precipitated, the active material could be thrown down by the addition of mercuric sulphate dissolved in 5 per cent. sulphuric acid. Up to this point the separation has been made repeatedly. After removing the Hg from the precipitate by H_2S, the activity of the preparation varies, probably with the amount of $HgSO_4$ used, and the concentration of H_2SO_4 in the mixture. The optimum conditions for precipitation have not yet been exactly determined.

Further purification of the precipitate, freed from mercury, has so far been unsatisfactory. Fractional crystallization leaves the active substance in a syrupy filtrate. Precipitation with $AgSO_4 + Ba(OH)_2$, of histidine and a syrupy material giving the histidine diazo test, leaves most of the active material in the filtrate. The addition of an excess of baryta causes some, but not all, to be thrown down together with impurities as the silver compound. Phosphotungstic acid apparently destroys the activity of the compound, although this requires further verification. It has not been possible to obtain active material either from the phosphotungstic precipitate or filtrate. The phosphotungstic acid has been removed both by baryta and by extracting with amyl alcohol and ether.

In attempting other methods, also, the activity has gradually diminished and been entirely lost, and it may prove impossible to obtain the material in pure form by methods at present available. It is hoped, however, that further work with larger quantities of material will result in the separation of this compound, which may prove to be of more general interest than simply from the standpoint of bacterial nutrition.

To sum up: the experiments here reported indicate that casein and certain other proteins contain a hitherto undescribed component, which also occurs in an infusion of beef and beef heart. It is essential to the growth of the hemolytic streptococcus and probably the pneumococcus, and is absorbed from the beef infusion by charcoal, and precipitated from the casein in an impure form by mercuric sulphate. The chemical nature of the substance has not yet been determined.

7 (1589)

The cause of the parallelism between the gram reaction and the gentian violet reaction.

By **JOHN W. CHURCHMAN.**

[*From the Laboratory of Bacteriology, Cornell Medical School, New York City.*]

In previous studies, published at intervals since 1912, it has been shown that a striking parallelism exists between the Gram reaction and the gentian violet reaction. The Gram positive organisms are killed by the stain and will not grow in agar containing it; the Gram negative organisms survive staining and grow vigorously in the presence of the dye. To this rule there are about 10 per cent. of exceptions.

Does this parallelism indicate that the two reactions have fundamentally the same explanation and that the power of the Gram positive organisms to fix the dye, so that it is retained in the Gram process, enables them also to fix it so that it leads to their death, or prevents their growth in media containing it? An attempt was made to answer this question by training a Gram positive organism (*B. subtilis*) to grow on agar containing gentian violet, working up gradually from minimal dilutions (1–1,000,000) to greater strengths. If a Gram positive organism so trained ceased to retain the stain by Gram's method the problem would be solved. This attempt was, however, wholly unsuccessful; it was impossible to train *B. subtilis* to grow in the presence of the dye.

A study of a Gram negative organism (*B. coli*)—which is also gentian negative—gave a partial answer to the question. If thick suspensions of this organism be stroked across a divided gentian violet plate, growth is equally vigorous on the two sides; the organism is apparently in no way restrained by the dye. If, however, instead of a thick suspension increasingly weak dilutions of the suspension be used for the stroking, the colonies on the gentian violet side of the plate become rapidly fewer as the dilution increases, and soon disappear altogether. The same result was obtained by similar experiments with other Gram negatives (*B. typhosus* and *B. prodigiosus*). That is to say: *In a thick suspension of a Gram negative organism only a small proportion of the individuals are Gentian negative; it is possible to isolate the Gentian positive individuals in pure culture, and when so isolated they are found to be as definitely Gram negative as the Gentian negative individuals. The factor which determines the reaction of an organism to the Gram process of staining is therefore not the same as the factor which determines its reaction on divided gentian violet plates, or after staining with the dye.*

8 (1590)

The isolation of gentian positive individuals from a suspension of a gentian negative organism (b. coli).

By JOHN W. CHURCHMAN.

[*From the Laboratory of Bacteriology, Cornell Medical School, New York City.*]

If divided gentian violet plates be stroked with increasingly weak dilutions of a suspension of the Gram negative and gentian negative *B. coli*, the organism will grow equally well on the two halves of the plate in the strokes made with strong dilutions, while in the strokes made with weak dilutions many more colonies will appear on the plain agar than on the gentian violet agar. If the dilution be very weak, in many instances no colonies whatever appear on the gentian violet side. This is due to the fact that only a relatively small proportion of the individuals, in a suspension of a Gram negative organism, are really gentian negative. If the suspension be thick this small proportion of individuals is, absolutely, sufficiently large in quantity to produce good growth in the presence of the dye. If, on the other hand, the suspension be weak, the gentian negative individuals are not only proportionally but absolutely few in number; few colonies therefore appear on the gentian violet agar; if the dilution be very weak none appear.

By cultivating the various colonies which appeared on the plain agar side of such a plate it has been possible to isolate from suspension of a gentian negative organism (*B. coli*) a gentian positive strain. *There may exist, that is to say, within a single bacterial strain, two types of individuals which, though in every other tinctorial and cultural characteristic identical, are quite dissimilar in their reaction to gentian violet, one growing vigorously and the other not at all on media containing this dye. These types retain the differential characteristic after many transplantations.*

9 (1591)

Relation of the gentian violet reaction to dilution of implanted suspension.

By **John W. Churchman.**

[*From the Laboratory of Bacteriology, Cornell Medical School, New York City.*]

It has been stated above that if increasingly weak dilutions of suspension of a Gram negative organism (*B. coli*) be stroked on a divided gentian violet plate relatively few colonies appear on the gentian violet side of the plate and, when very weak dilutions are used, none at all. It has been shown that this is, in part, due to the fact that in a suspension of a Gram negative organism by no means all, indeed, only a small proportion of the organisms may be gentian negative.

This can hardly, however, be the whole explanation. For if the experiment be repeated, using an emulsion of organisms which have already grown in the presence of gentian violet and have thus proven their resistance to the dye, a similar quantitative phenomenon is observed; far fewer colonies appear on the gentian violet agar than on the plain agar when weak dilutions of the suspension are stroked across the plate. The explanation of this fact is not clear; it may be due to some sort of communal property which enables bacteria, instead of pursuing individual careers, to aid each other in their growth and thus to accomplish in large groups what they cannot accomplish singly.

10 (1592)

The effect of repeated re-inoculations of gentian violet agar with gentian positive organisms.

By **John W. Churchman.**

[*From the Laboratory of Bacteriology, Cornell Medical School, New York City.*]

If a divided gentian violet plate be stroked with a thick suspension of the Gram positive and gentian positive *B. subtilis* no growth whatever will ever occur on the gentian violet side of the plate. The organism will, indeed, refuse to grow up to the dividing line between plain and gentian violet agar, ceasing sharply at a point about $^1/_2$ to 1 cm. from this line.

If, however, the gentian violet half of the plate be repeatedly re-inoculated with thick smears, a moderate growth—in some instances a fairly vigorous growth—occurs. This is not due to acquisition by the bacteria of resistance to the dye, for if the organisms, which have thus grown, be transplanted to gentian violet agar they will not grow in the presence of the dye. Nor is it due to insufficiency of dye, for in the plates used in the experiments dye was used in strengths greatly in excess (1–100,000) of the strength necessary to prevent the growth of *B. subtilis* (1–1,000,000).

The explanation of the phenomenon is not at present perfectly clear, but there is some reason for believing that it may be due to a property of bacteria—not hitherto recognized—of communal action. A few studies of single stained bacterial cells, made by the method of Barbour, lend support to this explanation.

11 (1593)

The selective action of gentian violet in relation to chemotherapy.

By **JOHN W. CHURCMHAN.**

[*From the Laboratory of Bacteriology, Cornell Medical School, New York City.*]

It is clear from the facts stated in the four previous communications that selective bacteriostasis is a complex process. The method of divided plates presents a perfect machinery for studying the workings of this process; and in the selective property of gentian violet we have a means of observing the various elements of the process under perfect control on a single plate. On such a plate we will find that

(*a*) Thick suspension of the Gram positive *B. subtilis* will not grow in the presence of the dye.

(*b*) By repeated re-inoculations of this organism a moderate growth can be procured on the gentian violet agar.

(*c*) Thick suspension of the Gram negative *B. coli* grow equally well on the two halves of the plate.

(*d*) If very weak dilutions of suspension of the Gram negative *B. coli* be stroked across a divided plate a few colonies appear on the plain agar, none at all on the gentian violet agar.

(*e*) From a suspension of the Gram negative *B. coli*, a gentian negative and a gentian positive strain can be cultivated.

(*f*) A thick suspension of the gentian negative strain of *B. coli* will grow equally well on the plain agar and on the gentian violet agar; if a weak dilution of the suspension be used a few colonies will appear on the plain agar, none at all on the gentian violet agar.

(*g*) If a thick suspension of the gentian positive strain of *B. coli* be stroked across the plate no growth whatever occurs on the gentian agar.

These observations indicate a number of the pitfalls which beset those who attempt to transfer laboratory observations into therapeutics. No conclusion as to the probable effect of a selective therapeutic agent is justified unless the experiments on which this conclusion is based have taken into consideration the quantities of bacteria used.

12 (1594)

The communal activity of bacteria.

By **JOHN W. CHURCHMAN.**

[*From the Loomis Laboratory, Cornell Medical School, New York City.*]

It has been shown above that, while *B. coli* like—most of the gram negative organisms—is apparently uninfluenced in growth by gentian violet, a careful study of thick suspension will demonstrate the presence of many individuals which *are* susceptible to the dye.

The isolation of a strain of *B. coli* entirely fast to gentian violet—that is to say, containing no individuals susceptible to the bacteriostatic effects of the dye—has made it possible to study quantitatively the reaction between this bacteriostatic agent and bacteria, without encountering the disturbing factor usually met in such studies and caused by the variability of the susceptibility of the individual organisms to the chemical substance under examination. This strain of *B. coli* had been isolated from a single colony growing on gentian violet agar and had been kept growing on gentian violet agar by frequent transplants over a period of several weeks. Every individual had therefore proven its ability to grow in the presence of the dye by actually having done so.

Working with such a culture it is found that, though large inoculations of gentian violet media produce as heavy growths as in plain media and that the dye therefore seems to have no inhibitory effect, single cell transplantations (by the method of Barbour) never grow. Nor does growth occur if small groups (under 30) are transplanted. *This would indicate that bacteria do not, as is commonly supposed, act as isolated individuals; they possess the power, in numbers, of accomplishing effects which, alone, they are incapable of. The nature of this community of action it is at present impossible even to guess at.*

13 (1595)

Resistance of hepatic tissues to local anemia.

By **LOREN R. CHANDLER** (by invitation).

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

If a temporary renal anemia of two hours' duration is produced by placing a ligature about the renal artery of a rabbit, and the rabbit is killed from one to four days later, histological study will invariably show necrosis of practically the entire cortical tubular epithelium, with few if any changes in the glomerular and interstitial elements. This method of producing epithelial necrosis, with the minimum amount of injury to other elements, is now being used in this laboratory for a study of epithelial regeneration and the pathology of renal excretion.

As a preliminary to a similar study of hepatic function and regeneration, tests were made of the effects of temporary local anemia on the liver of dogs. To produce this anemia, Eck fistulas were made. From five to seven weeks later the abdomen of each dog was reopened, and temporary ligatures placed about the hepatic artery and portal vein. The ligatures were kept in place for from three to twelve hours. The animals were killed from two to six days later.

During the period of ligation, the dogs showed no toxic symptoms. After the release of the ligatures they were in every way apparently normal till the date of the autopsy. The following is a summary of the histological findings:

(*a*) *Three hour anemias:* No thrombosis. No necrosis or atrophy of the hepatic parenchyma. Moderate degree of fatty degeneration, mainly confined to the central third of the lobule.

(*b*) *Twelve hour anemias:* No thrombosis. No necrosis. Marked fatty degeneration of the central half of the lobule, with slight atrophy of the parenchyma immediately surrounding the central vein.

From these findings we conclude that the almost total anemia produced by temporary ligation of the hepatic artery and portal vein in Eck fistula dogs, for periods as long as twelve hours, does not cause necrosis of the hepatic parenchyma.

This power of the hepatic cells to resist local anemia probably accounts in large measure for the infrequency of infarcts in the liver, which infrequency is usually attributed solely to the presence of the double hepatic circulation.

Experiments extending over longer periods of time will be reported later.

14 (1596)

An attempt to produce hemochromatosis experimentally.

By **Loren R. Chandler** (by invitation).

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

The hypothesis is suggested by MacCallum[1] that hemochromatosis may possibly be due to iron retention, secondary to decreased excretion of waste iron by the colon. We have attempted to test this hypothesis by a surgical removal of the colon of dogs.

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[1] MacCallum, "Text-Book of Pathology," 1916, p. 112.

This removal was performed in two stages. First, the entire large intestine was separated from the mesentery by means of an abdominal incision, and the abdomen closed. The entire colon was then withdrawn through the anal opening, by a modified Whitehead operation, care being taken not to injure the anal sphincters. The end of the ileum was sutured to the anal mucosa.

After the operation, the dogs were kept on a milk diet for about a week, and then placed on an ordinary mixed diet. Most of the dogs died from shock or intercurrent infections, or were killed for pathological study at the end of from one to two weeks. One dog, however, was kept for three-and-a-half months.

This dog showed a rapid loss of weight during the first two weeks following the operation, after which it slowly gained in weight till the end of the experiment. The dog apparently suffered no inconvenience from the operation, other than that from the frequent passage of semi-liquid stools.

At autopsy this dog showed no pigmentation of the internal organs that could be detected macroscopically. Frozen sections and celloidin sections of the spleen, liver, pancreas, small intestine, kidney, bone-marrow and heart muscle showed no pigment deposits. No iron-containing pigment could be detected in these organs by the Berlin blue reaction.

The total removal of the large intestine in dogs, therefore, apparently does not produce a recognizable degree of haemochromatosis within a period of three-and-a-half months.

Experiments extending over a longer period of time will be reported later.

15 (1597)

Variations in the total cholesterol content of the blood serum in pernicious anaemia and pneumonia.

By **H. A. KIPP** (by invitation).

[*From the Laboratory of Pathology, University of Pittsburgh.*]

Pernicious anemia and pneumonia are pathological states in which the cholesterol content of the blood is known to be altered during the course of the disease. In pernicious anemia, the cholesterol content of the serum is depressed to a varying degree below normal, paralleling the severity of symptoms, the diminution in the erythrocyte content and hemoglobin percentage of the blood. In view of the fact that cholesterol has been shown to possess the property of neutralizing the hemolytic action of various materials, animal and vegetable toxins, an increase in the severity of symptoms in pernicious anemia with the depression of this substance in the blood serum suggests the utilization of this antitoxic property against the unknown hemolytic toxins which are present in the body in this disease.

The transfusion of whole blood from a donor whose serum cholesterol content was relatively high, was apparently without permanent influence on the cholesterol content of the serum of the pernicious anaemia patient, regardless of the clinical improvement which was temporarily manifest. It may be that the additional cholesterol added in the infused blood contributes in a degree to the temporary clinical improvement of the patient, although the cholesterol level in the blood serum is maintained at a low figure and is again lowered with a relapse of the symptoms.

In pneumonia, the cholesterol in the serum was found to follow a variation dependent upon the severity of the disease, the amount of involvement of the lung tissue and development of the pneumonic exudate in the inflammatory process which follows the bacterial invasion. There is a primary depression of the cholesterol content of the serum in the first few days of the disease. This depression seems to be dependent upon the severity of the disease and particularly upon the degree of involvement of the lung tissue by the inflammatory process. With the development of an empyema, the cholesterol content of the serum remains lowered until this process of inflammation is resolved. With the convalescence of the patient and particularly with resolution of the exudate in the lung, there is a rise in the cholesterol content of the serum which amounts to a hypercholesterinaemia. This rise apparently parallels the resolution of the inflammatory exudate which has been shown to contain a considerable amount of cholesterol. The variation in the cholesterol in serum parallels the activity of the phagocytic leucocytes and owing to its colloidal nature and consequent relative indiffusibility, the transportion of the cholesterol to the area of inflammation is dependent upon the leucocytes which, as pus cells, have been shown to contain a considerably greater amount of cholesterol than the normal leucocytes.

Analysis of empyema exudates showed that the greater part of the cholesterol is contained in the cellular portion of the exudate. The fluid portion of exudates contains even less cholesterol than normal serum or exudates in the pleural cavity which contain relatively few cells.

Since the activity of the leucocytes is an important factor in the resistance to and recovery from pneumonia, the association of the variation of the cholesterol with the activity of the leucocytes seems to indicate the rôle which cholesterol plays on leaving the blood serum in acute infections. Carried by the leucocytes to the site of the active inflammatory process, cholesterol is available for the neutralization of bacterial toxins and poisons arising from the disintegration of tissue and exudate in the process of resolution of the pneumonic exudate.

16 (1598)

Effect of antipyretics on memory and behavior of albino rats.

By **D. I. MACHT** and **WM. BLOOM.**

[*From the Pharmacological Laboratory, Johns Hopkins University.*]

Studies were made by the authors on the behavior of white rats in Watson's circular maze. All the drugs were administered by subcutaneous or intraperitoneal injections. The following drugs were studied; acetanilid, acetphenetidin, antipyrin, pyramidon, sodium salicylate, phenyl salicylate or salol and quinine sulphate.

Acetanilid was administered in doses from 1 to 5 milligrams and was found to produce depression. Phenacetin also produced depression but not to the same extent. Salol in small doses produced no effect; larger doses (5 milligrams) caused slight depression. Sodium salicylate caused slight depression. Quinine produced depression when administered in doses from $2^1/_2$ to 5 milligrams. Antipyrin was found to be most depressing of all even when the doses were 2 milligrams. Pyramidon was also depressing but not to the same extent.

The following combinations were studied: acetanilid plus sodium bicarbonate, acetanilid plus phenacetin, sodium salicylate plus salol, phenacetin plus pyramidon, acetanilid plus pyramidon and salol plus acetanilid.

It was found that acetanilid plus phenacetin and salol plus sodium salicylate combinations gave a summation effect, whereas phenacetin plus pyramidon and acetanilid plus pyramidon exhibited synergistic phenomena. The most striking combinations were acetanilid plus bicarbonate of soda and acetanilid plus salol. In the case of each of these combinations acetanilid was not as depressent as when given alone.

The effect of opiates on the behavior of rats has already been published. Investigations are in the process of completion concerning the effect of the following drugs on the memory and behavior of rats in the maze: alcohol, caffeine and nicotine; cocaine and its decomposition products; digitaloid drugs and some others. Complete data concerning the antipyretics will appear in the *Journal of Pharmacology and Experimental Therapeutics.*

17 (1599)

Amicronucleate infusoria.

By **LORANDE LOSS WOODRUFF.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

It has generally been accepted that the dimorphic condition of the nucleus (macronucleus and micronucleus) is a diagnostic character of typical Infusoria, and, aside from a few primitive or aberrant species, the only apparent exceptions have revealed the micronucleus (or micronuclei) within the macronuclear membrane during vegetative stages. Recently, however, Dr. Dawson, working in this laboratory, described a race of Oxytricha hymenostoma Stokes which throughout several years of pedigree culture showed no indication of a morphological micronucleus.[1]

During the past year, the isolation for certain experiments of 14 "wild" lines representing 6 species of hypotrichous Ciliates revealed 7 lines (4 species) with micronuclei and 7 lines (2 species) without morphological micronuclei. Ten of the lines were all isolated from a "wild" mass culture of the same species, *Urostyla grandis*, found in a laboratory aquarium. Six of these lines were amicronucleate. All of the lines of all of the species have bred true with respect to the character in question and one amicronucleate line at present is at the 102d generation.

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[1] J. A. Dawson, "An Experimental Study of an Amicronucleate Oxytricha," *Journ. Exper. Zoölogy*, 1919, xxix, 473; 1920, xxx, 129.

Similarly a culture of *Paramecium caudatum*, which the present writer supplied a year ago to a course in protozoölogy for the study of the nucleus, failed to reveal a micronucleus, although in other races the micronucleus was readily demonstrated.

The apparent conclusion is that a distinct morphological micronucleus is a variable character among different races of the common free-living Ciliates and this, obviously, leads to many interesting problems in relation to conjugation and endomixis.[1]

18 (1600)

A preliminary report on the experimental production of sarcoma of the liver of rats.

By F. D. BULLOCK, M. R. CURTIS, and G. L. ROHDENBURG.

[*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.*]

The association of sarcoma of the liver of rats with *Cysticercus fasciolaris*, the larval stage of *Tenia crassicollis* of the cat, has been noted by a number of investigators, including two of the present authors; but to our knowledge no one has hitherto reported the experimental production of tumors by the employment of this parasite as an agent. The purpose of the present note is to record several cases of sarcoma of the liver in a group of 500 rats infested with the *Cysticercus* by feeding the animals eggs of the *Tenia* obtained from cat feces. Two hundred and fifty of these rats were alive when the first tumor was discovered, and 170 are still under observation.

Large tumors were discovered in the livers of four rats, 296 to 357 days after feeding. In each case the tumor originated in the wall of a single *Cysticercus* cyst, one of 30 to 50 present in this organ. Each of the involved cysts contained a worm about 20 cm. long, only one of which was living. Three of the tumors had metastasized freely into the peritoneal tissues. In each of two of the animals early and probably independent malignant changes had occurred in the walls of other cysts in the liver. Histologically, the tumors were sarcomata of either the spindle-cell or polymorphous-cell type. The transplantation of two of them into young rats resulted in 92 and 46 per cent. respectively of successful inoculations. The other two were not transplanted.

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[1] E. M. Landis announces in the current number of the American Naturalist, Vol. 54, pp. 453–57, the discovery of an amicronucleate race of *Paramecium caudatum.*

Complete data on a fifth rat which bore a tumor is lacking due to the loss of the liver through partial evisceration of the animal by his cage mates. The peritoneal tissues were, however, studded with tumor nodules which histologically proved to be spindle-cell sarcoma. In all probability these nodules were metastases from a primary growth of the liver.

19 (1601)

Further studies on intestinal implantation of bacillus acidophilus.

By **Harry A. Cheplin** and **Leo F. Rettger.**

[*From the Sheffield Laboratory of Bacteriology, Yale University, New Haven, Conn.*]

The more recent observations on transformation of the intestinal flora in man have fully confirmed the earlier conclusions, which are briefly summed up as follows. The daily administration of 150–300 grams of lactose or dextrin to adults will, with few exceptions, bring about a marked change in the character of the flora in which the usual mixed types of bacteria give way to *Bacillus acidophilus* of Moro, which is a normal intestinal organism, but which is present in the intestine after early infancy in relatively small numbers only. In some instances 350–400 grams of the carbohydrates are required. The same results may be brought about with 150–300 cubic centimeters of a whey broth culture of *B. acidophilus* and with 500–1,000 c.c. of *B. acidophilus* milk, as well as with smaller amounts of the milk in combination with 100 grams of either lactose or dextrin.

Particular attention is being given to the preparation of *B. acidophilus* milk which is uniform from day to day in its physical and chemical properties. Experience thus far has shown that such a product may be obtained easily when certain conditions are carried out. In the first place, the stock strains, preferably mixed strains, must be grown sufficiently long in milk to bring about light curdling within a period of 24 hours. When such strains are once developed they should remain viable for many months at least. We are still employing the strains which were first used for this purpose eight months ago, and are unable as yet to detect any signs of deterioration. The character of the soft curd is very much influenced by the quality of the milk at the time of sterilization preliminary to inoculation. If the milk is more or less acid, even though no curdling is observed, the final product tends to be of uneven consistency, granules and lumps of curd being quite apparent. As a rule overnight incubation suffices to bring about the formation of the soft curd. At any rate, the incubation should not be continued for more than 24 hours.

Successfully prepared *B. acidophilus* milk should have the following properties. It should be of a uniform creamy consistency, except for some particles of thin film which is formed during the process of sterilization of the milk, and should have only a very thin layer of whey on the surface. The creamy character becomes more marked on vigorous shaking of the product. The odor should be slightly aromatic with no suggestion of ordinary bacterial decomposition. Both odor and taste should be pleasant. On standing at ordinary or refrigerator temperature without contamination, little change should be noted in the physical and chemical characters. The acidity, which always remains well below 1 per cent., increases but little after the initial incubation period. Samples of the milk which have been held at room temperature for two weeks were practically indistinguishable from the freshly prepared product.

Although a number of clinical cases have been included in the 30 or more subjects which have been employed in the present study of implantation of *B. acidophilus*, and with most promising results, we do not wish to make any statements as to the therapeutic value of *B. acidophilus* feeding until a time when an abundance of information on this phase may be at hand.

A full record of the authors' work is now in the Yale University Press, and will appear in book form at an early date.

20 (1602)

Changes in organ weight produced by diets deficient in antiscorbutic vitamine.

By **VICTOR K. LAMER** and **H. L. CAMPBELL.**

[*From Columbia University, Dept. of Chemistry, New York City.*]

Young guinea pigs weighing 250–300 grams, fed on a diet deficient in water-soluble C (antiscorbutic vitamine), show at death a pronounced increase in weight of the adrenal glands amounting to approximately 100 per cent. when computed on basis of body weight minus alimentary canal. (Confirming McCarrison's statement.)

The increase in size is equally definite but not so pronounced when computed on basis of the beginning, or maximum, body weight attained. Starvation controls do not show an increase in adrenal weight.

The increase in adrenal weight is directly proportional to the length of time which the animal is on the scorbutic diet and is most pronounced in those animals in which life has been prolonged by affording them partial protection with small but insufficient quantities of tomato juice.

This may be interpreted as indicating a compensatory response to the decreased adrenalin production known to exist in the scorbutic animal. This point is of interest in connection with the extensive intramuscular and intestinal hemorrhages found in scurvy.

Our data comprising 40 scorbutic and 15 control animals gives no indication that the liver is affected by a lack of water-soluble C alone. There is, however, some evidence that the heart and kidneys are increased on the scorbutic diets.

ABSRACTS OF THE COMMUNICATIONS,

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Twenty-sixth meeting.

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21 (1603)

Experimental bronchopneumonia and empyema in the rabbit.

By **F. P. GAY** and **BERNICE RHODES.**

[*From the Department of Pathology and Bacteriology, University of California.*]

Gay and Stone have described an experimental streptococcus empyema in rabbits which presents advantages for the study of preventive and curative measures against this condition. It resembles in all details human streptococcus empyema in that it is a process of infection by extension involving not only the side of the chest inoculated but the pericardium and the other pleural cavity. The infection apparently becomes septicemic only in its terminal stages. This experimental syndrome as produced by inoculating into the pleural cavity of rabbits differs from the human process only in its method of origin which in man is by an extension of the streptococcus down the respiratory tract with more or less involvement of the lungs in the form of a bronchopneumonia.

Our early attempts to produce streptococcus pneumonia in rabbits were unsuccessful owing, we believe, to the fact that we employed a culture of a streptococcus that had not been passed through the pleura of rabbits as is the one we now uniformly employ to produce empyema by intrapleural injections. And secondly, in our earlier attempts the culture was injected between the cartilages of the trachea by means of a hypodermic needle. We have now succeeded in producing bronchopneumonia and empyema by means of our passage streptococcus culture, grown in blood broth and injected into the trachea through a catheter in the manner described by Winternitz and Hirschfelder, followed by forcible insufflation with air. This method of injection is difficult, but in the four animals that we have injected in this manner and which have died or been killed in from one to five days, definite consolidation of the lungs was evident in all and a sero-fibrino-purulent pleurisy occurred in all but the 24-hour case.

A histology study of the lungs in these cases apparently shows the characteristics described by MacCallum in his interstitial broncho-pneumonia, namely, plugging of aveoli with polymorphonuclear leucocytes, red blood corpuscles, serum and fibrin in definite relation to bronchi which are also filled with a purulent exudate. There is a definite infiltration of polymorphonuclear leucocytes and lymphocytes about the bronchi and blood vessels and marked desquamation of the bronchial epithelium. A further study will show in what respect, if any, this experimental pneumonia in rabbits differs from that produced by the pneumococcus.

22 (1604)

The bactericidal action of rabbit bile on certain strains of streptococci.

By **Ruth L. Stone** (by invitation).

[*From the Department of Pathology and Bacteriology, University of California, Berkeley, California.*]

The phenomenon here described was noted during the course of a series of experiments on rabbits designed to test the pathogenicity of a certain strain of hemolytic streptococcus. It was found that, although at autopsy the various organs of the peritoneal cavity were filled with living streptococci, the bile was always sterile. This led to the testing, in vitro, of bile from other rabbits as well as from various other animals, to find out, whether they possessed bactericidal action on this strain of streptococcus. All samples of rabbit bile proved to be bactericidal, whereas the bile of the ox, sheep, cat, dog, pig, guinea pig, and human exerted no deleterious effect on the streptococci.

The strain of streptoccus used (Strain "H")[1] in these preliminary experiments was, according to Holman's classification, *Streptococcus pyogenes*—a hemolytic, non-mannite fermenting streptococcus. Our next step was to test various strains of streptococci from human and animal sources, these strains having been classified according to their hemolytic and sugar fermenting properties.

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[1] Gay and Stone, *J. Infec. Dis.*, 1920, xxvi, 265.

The results may be briefly summarized, by dividing the organisms into three groups, at least two of which are apparently clear cut.

I. All those hemolytic non-mannite fermenting *Streptococci* which fall, by Holman's classification, into the *Streptococcus pyogenes* group, were killed by rabbit bile, 1/50 of 1 c.c. of bile, or less being sufficient to kill 0.1 c.c. of a 24-hr. serum broth culture. About twenty cultures of this type were tested.

II. All non-hemolytic *Streptococci*, whether of human or bovine origin, were unaffected by rabbit bile.

III. Hemolytic, mannite fermenting streptococci are *almost* always unaffected by rabbit bile. In a group of thirty or more of such strains tested, only two were killed by bile.

Since this bactericidal power of rabbit bile is undiminished by sterilization, attempts were made, by fractioning the bile, to determine, if possible, what constituent of rabbit bile is responsible for this highly selective bactericidal action.

Bile was dried with sand, to give greater surface for extraction, and the resulting mixture ground and treated with absolute alcohol, thus precipitating the proteins. The resulting filtrate was evaporated to dryness and then resuspended in broth to the original volume of the bile. This was sterilized and tested for its bactericidal power, which was found to be undiminished. Next, a portion of this alcoholic extract was treated with absolute ether, causing a further precipitate. Both filtrate and precipitate were dried and resuspended in broth, and tested as before. It was found that only the precipitate contained this bactericidal substance. It may be of interest here to note that Neufeld found that the pneumococcus dissolving substance of bile was also located in this fraction. However he found this to be true of various types of bile, whereas the phenomenon here described only occurs with rabbit bile, and is a bactericidal and not a lytic process, since the bacterial bodies are visibly intact even after 48 hours.

On treating the alcohol soluble fraction with acetone, both filtrate and precipitate were found to be slightly bactericidal, but neither equal to the original power of the alcoholic extract.

It is evident, therefore, that this bactericidal substance in rabbit bile for certain strains of streptococci, is present with or identical with a bile salt, being precipitated by ether, and alcohol soluble. However, since other types of bile do not give these reactions which seem to be peculiar to rabbit bile, one must conclude that rabbit bile either has some substance in its composition that is not found in other types of bile, or that its chemical construction is different, thereby giving it this peculiar property.

23 (1605)

The viability of B. typhosus in alkaline bile in vivo.

By **T. D. BECKWITH** (by invitation).

[*From the Department of Pathology and Bacteriology, University of California.*]

In as much as Nichols suggests the use of alkaline therapy for the purpose of eradicating *B. typhosus* within the gall bladder of human carriers of the disease, the following observations are pertinent.

While carrying out a series of tests with experimental rabbit carriers of typhoid, it was noted in a certain instance that the hydrogen ion concentration of the bile was different from that supposed to characterize the normal animal. This indication was followed with other animals as opportunity presented itself. P_H determinations were made on the bile of uninfected animals as materials appeared. The method followed was that of Clark[1] and Lubs with the comparator block introduced into the system. Readings were made as soon after the death of the animal by exsanguination as possible, generally within three quarters of an hour. In order that contact with the air and consequent loss of dissolved gases might be reduced to a minimum, the bile was kept either within the closed syringe with which it had been aspirated or was placed within a small bore agglutination tube. All animals had been fed regularly on a ration of rolled barley and succulent grass.

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[1] Clark, W. M. and Lubs, H. A., *Jour. Bact.*, 1917, ii, 1–34, 109–136, 191–236.

No animal was classed as a carrier unless it had been inoculated with *B. typhosus* at least two weeks previously since it was felt that time sufficient for physiological adjustment should be given. This period is arbitrary but is conservative since between one and two weeks are required before the weight curve of the animal commences to ascend. Rabbit carriers were prepared according to the method of Gay[1] and Claypole[2] which in this series yielded 100 per cent. efficiency. No bile was classed as having been taken from a positive carrier unless subsequently *B. typhosus* was isolated from it and confirmation made by agglutination.

A series of nine rabbit biles from experimental carriers yielded a mean hydrogen ion determination by the colorometric method of P_H 8.33. A check series of twenty-seven units taken from normal animals gave a mean reading of P_H 7.41. The factor of variability for the first series taken from carrier animals is 6.4 per cent. while the like factor from the larger check series is 2.3 per cent. The two therefore are comparable.

No reason thus far is assigned for this difference in P_H of the two series of fluids. It is felt that a possible explanation based on lysis of cells in the course of the inflammatory process with increase in alkalinity is not sufficient since the lowered hydrogen ion concentration which may be expected to result should be expected to occur earlier were it the cause. The lowered P_H was most evident ten days following preparatory injection of the typhoid organisms.

The results obtained for normal rabbit bile coincide with those of Quagliariello[3] but are somewhat higher in the concentration of the hydrogen ion than those given by Okada[4] who however was working with hepatic rather than with cystic bile.

Relative to these observations it may be stated that *B. typhosus* will live for at least 24 hours in ox bile the reaction of which after autoclaving is altered to P_H 9.2 by the addition of an appropriate amount of sodium hydroxide, the incubation temperature being 37° C.

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[1] Gay, F. P. and Claypole, E. J., *Archiv. Int. Med.*, 1913, xii, 613.

[2] Nichols, H. J., *Jour. Exp. Med.*, 1916, xxiv, 495–514.

[3] Quagliariello, G., *Atti d. Reale Accad. d. Lincei*, 1911, xx, Ser. 5a, 302–305.

[4] Okada, S., *Jour. Phys.*, 1915, l, 114–118.

Results: (1) Bile from carrier rabbits of *B. typhosus* shows a lower hydrogen ion concentration than that from normal animals. In the first the general mean was 8.33 while the latter gave 7.41.

(2) *B. typhosus* is viable in vivo in rabbit bile even when the hydrogen ion concentration is depressed to P_H 9.4.

24 (1606)

The conditions under which the ratio between the urea content of the urine and of the blood remain constant.

By **T. Addis** and **D. R. Drury.**

[*From the Laboratory of the Medical Division of Stanford University Medical School, San Francisco.*]

Simultaneous measurements of the hourly rate of urea excretion and of the blood urea concentration have been made under various conditions in man in order to determine whether the ratio between the urine and blood urea ever becomes approximately constant. After the administration of urea and large amounts of water the ratio shows at first a considerable variability, but after the maximum urea concentration in the blood has been attained and the concentration is slowly falling the ratio becomes constant for each individual within fairly narrow limits. Food, excitement, and the various other factors produce marked variations even under these conditions. We have not been able to confirm the conclusion of Austin, Stillman and Van Slyke, that the ratio of urea excretion varies with the square root of the volume of urine when the blood urea concentration is constant.

Under the special conditions outlined above, the rate varies directly with changes in the blood urea concentration from 118 to 20 mgs. per 100 cc.

25 (1607)

The relation of fodder to the antiscorbutic potency and salt content of milk.

By **ALFRED F. HESS, L. J. UNGER** and **G. C. SUPPLEE.**

[*From the Department of Health, New York City.*]

Five Holstein cows which had been stall-fed throughout the winter were given for a period of three weeks fodder containing practically no antiscorbutic vitamine. The dietary consisted of 25 pounds of a concentrate mixture composed of one part of bean meal, two parts of pressed flaxseed oil meal, two parts hominy, two of gluten meal and two of bran; each received also 8 pounds of kiln-dried beet pulp, 4 quarts of molasses and 12 pounds of straw. On this diet the milk flow decreased at first 10 pounds and later 5 pounds a day. The milk at the end of this period was dried by Just hot-roller process.

The five cows were then put out to pasture for three weeks, and a day's sample collected and dried in the same way.

Feeding experiments with guinea pigs showed that the "dry fodder" milk was almost devoid of antiscorbutic potency, whereas the "pasture milk" was rich in this factor, although the cows had been on fresh food but three weeks.

The salt content of the two lots of milk also showed marked differences; that secreted on the "antiscorbutic free fodder" was considerably lower in calcium, phosphorus and citric acid, but contained a higher percentage of sulphur (calcium oxide .138 and .165, phosphorus pentoxide .158 and .190, citric acid .08 and .13, sulphur .023 and .014).

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

One hundred tenth meeting.

New York Post Graduate Medical School, New York City, November 17, 1920. President Calkins in the chair.

25 (1607)

Preliminary experiments with the fat-soluble vitamine (vitamin A).

By **H. C. SHERMAN**, **F. L. MACLEOD** and **M. M. KRAMER.**

[*From the Laboratory of Food Chemistry, Columbia University.*]

The term "fat soluble vitamine" or "vitamin A" is here employed to designate the substance or substances occurring in butter fat, egg fat, codliver oil and elsewhere by virtue of which growth is promoted when the diet is otherwise adequate, and the characteristic eye disease, noticed especially in rats by Osborne and Mendel, is prevented and may often be cured. If, as indicated by some recent observations, especially those of Hess, the relations of butter fat and codliver oil to rickets are so different as to suggest that their vitamines are different substances, it becomes conceivable that more than one substance having growth-promoting and "antixerophthalmic" properties may be embraced under the one term "fat-soluble vitamine" or "vitamin A" as now used.

1. *Distribution of the Substance or Substances "Vitamin A" between the Fatty and Aqueous Phases in Milk.*—Several years ago McCollum stated in a brief note that fat soluble A is about thirty times more soluble in fat than in water, in which case skimmed milk will contain about half as much of this vitamine as whole milk. On the other hand, Mellanby, studying experimental rickets in puppies, and Hess and Unger in their studies of the clinical rôle of the fat soluble vitamine, appear to have assumed that their experimental diets could contain considerable amounts

of skimmed milk, either in fluid or solid form, and still be nearly devoid of the fat soluble vitamine. According to our experience, skimmed milk contains a very significant amount of fat soluble vitamine, probably about half as much as whole milk as McCollum's brief statement would imply.

Our experimental evidence of the presence of significant amounts of fat soluble vitamine or "vitamin A" in skimmed milk is two-fold. (1) Young rats placed at weaning upon a diet in which dried skimmed milk was the sole source of vitamines have grown steadily (though at less than the maximum rate) for three months or more, trebling their body weights and remaining free from eye disease and in good general condition. Such results in rats of this age can be obtained only on diets furnishing significant amounts of "vitamin A." (2) Rats which had been brought to the typical condition of declining body weight and characteristic eye disease due to deficiency of fat soluble vitamine in their food have been cured by the feeding of skimmed milk powder. [A third type of experiment may be mentioned which, while it would not be conclusive alone, affords interesting confirmation. Rats which had failed to grow upon a diet of white bread, grew with extraordinary rapidity for some time (though not to full adult size) when the bread was supplemented by dried skimmed milk only. The latter of course supplemented the bread in several ways, but unless the skimmed milk had furnished important amounts of fat soluble vitamine such rapid and extensive growth would hardly have been possible.]

2. *Fat-soluble Vitamine in Growth and Reproduction.*—The results of our experiments are entirely consistent with previous findings that fat soluble vitamine is essential to growth and indicate further that the proportion of this vitamine in the food may have quite as striking an influence upon reproduction as upon growth.

3. *Storage of Fat-soluble Vitamine in the Body.*—While a moderate concentration of this vitamine in a diet excellent in all other respects suffices to support normal growth, and a more liberal supply of the vitamine may not then affect the growth rate, it appears that a surplus of the vitamine above the immediate needs of growth or maintenance may be largely stored in the body and subsequently utilized to meet the needs of reproduction and

lactation, or to carry the animal over a period of subsistence upon foods deficient in this vitamine. Animals kept under like conditions upon the same diet deficient in fat soluble vitamine show different survival periods according as their previous diet was rich or poor in this substance. Our results suggest that it may be largely because of previous storage of this vitamine that adult animals seem less dependent upon it than do young of the same species.

4. *Heat Destruction of the Fat Soluble Vitamine.*—Dry heating at a temperature of 100° C. with free access of air, only very slowly destroyed fat soluble vitamine. The results thus far obtained emphasize the importance of taking full account of the time as well as the temperature of heating, and of the initial concentration of the vitamine in the food, as well as of the opportunity for previous storage of the vitamine by the test animal.

Experiments upon these four problems are being continued.

26 (1608)

A new diet for gastric ulcer.

By **WARREN COLEMAN.**

[*Department of Medicine, University and Bellevue Hospital Medical College, New York City.*]

From the dietetic standpoint the two main indications for the treatment of gastric ulcer are:

1. To protect the ulcer from mechanical and chemical injury, and

2. To maintain the nutrition of the patient at a level which will favor the healing of the ulcer.

In the author's opinion, none of the diets hitherto employed in the treatment of gastric ulcer fulfills these indications: in none of them do the therapeutic properties of food appear to have received adequate consideration. In general, the frequent administration of small or moderate quantities of milk constitutes the basis of diets for gastric ulcer. Even admitting the economic quality of the secretion called forth by milk, its hourly or two-hourly administration keeps the secretory glands of the stomach in a state of constant activity. Recognizing this some authors advise the neutralization of the gastric contents with magnesia or soda midway between the feedings. Since the majority of the symptoms of gastric ulcer, at least in the earlier stages, and possibly considerable injury, are referrable to hyperacidity, such a diet lacks physiological support. In addition to excessive secretion, the motor activity of the stomach is abnormally increased.

In the diet which I propose the objections referred to have been eliminated. Moreover, I believe that the maximum protection of the ulcer obtainable when food is given by mouth has been achieved. As in some other diets, complete rest is given the stomach for 3 to 5 days through the use of glucose enemata—300 c.c. of a 7 per cent. to 12 per cent. solution by the Murphy-drip method, three or four times a day. The quantity of water is sufficient to prevent thirst. Protein and fat are not added to the enemata because of the doubt of their absorption.

Since a satisfactory state of nutrition cannot be maintained by nutrient enemata alone, foods for administration by mouth must be selected. Theoretically, egg-albumin to furnish the necessary nitrogen and olive oil to furnish the greater part of the energy most nearly meet the requirements. According to Pawlow egg-albumin, alone, does not call forth gastric secretion and oils inhibit it. In addition, olive oil affords mechanical protection to the ulcer. The olive oil is given at first in moderate but gradually increasing quantities, up to a total of 150 c.c. a day—1,395 calories. The whites of two or three eggs a day are added shortly after the oil is begun and increased to five or six—7–8 gm.N and 450 calories. The 100 gm. of glucose, given by rectum throughout the treatment, brings the energy intake to some 2,200 calories a day. The procedure is continued for three or four weeks. This diet has been employed for about eight years and the results have been satisfactory.

27 (1609)

Tests for physiological differences in transplantable tumors.

By L. C. STRONG and C. C. LITTLE.

[*From the Carnegie Institution of Washington.*]

The purpose of this paper is to present a method whereby two neoplasms, that are histologically identical, can be shown, nevertheless, to be different in their physiological reactions. This experiment was begun June, 1920, and is still being continued. Enough data have been accumulated, however, to warrant a preliminary report.

The tumors employed are two adenocarcinomas of the mammary gland, that arose spontaneously in two female mice of a closely inbred strain, the second one arising some three weeks after the first. The mice have been rigidly inbred, brother to sister matings, for about eleven years. One would expect, in that time, that the strain must have become homozygous in all, or nearly all, genetic factors that no doubt underlie morphological and physiological characters. This conclusion is warranted by evidence obtained from implanting bits of the two tumors into mice of this strain. The trochar method of implanting the neoplastic tissue has been employed throughout the experiment. In this preliminary experiment both tumors grew in 100 per cent. of all mice inoculated, irrespective of whether the two tumors were inoculated into the same mouse or into separate individuals. In case the two were inoculated into the same animal, the first (dBrA) was always inoculated into the right axilla, the second (dBrB) into the left axilla. The growth curves for each were charted from weekly observations. There was apparently no effect of one tumor upon the other when growing in the same mouse, either in percentage of indications or rate of growth. They remained entirely distinct. The question naturally arose, "Are the two tumors actually identical?"

The complexity of the genetic factor system that evidently underlies susceptibility to transplantable tumors makes it highly improbable that two *identical* tumors could arise independently within three weeks of each other, even in the same relatively homozygous strain of mice.

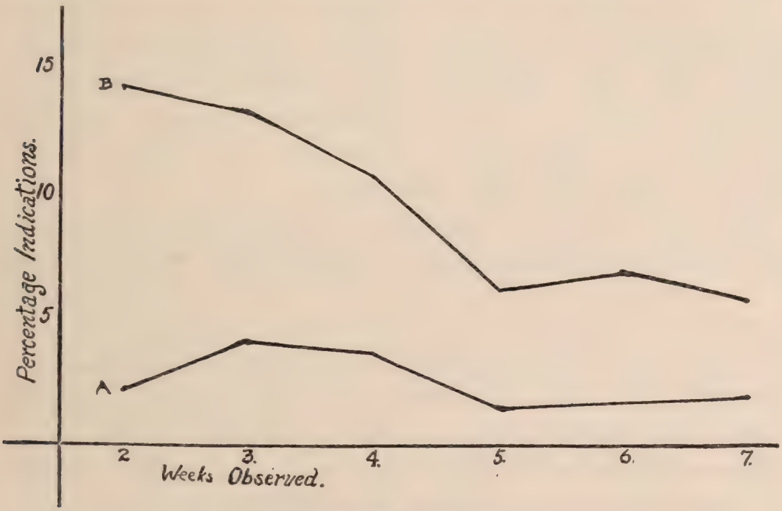
With the hope of discovering a strain of mice that would be susceptible to the dBrA tumor and not to the dBrB, our first choice was the wild house mouse, collected in several localities near the laboratory at Cold Spring Harbor, N. Y.

The first and foremost consideration in an experiment with transplantable tumors is the selection of the strain of mice to be used. Race has been recognized by many investigators in this field as a factor underlying tumor susceptibility and yet it is the one most often ignored. Several investigators have explained their fluctuating results as due to variations in the tumor cell whereas the same results can be explained by assuming fluctuations, although slight, in the strain of mice employed. This last explanation, certain to apply in most cases, is the only one acceptable to present-day geneticists without positive histological proof of tumor-cell modifiability. Racial homozygosity can only be obtained by rigid inbreeding, brother to sister matings, for several generations. The time element necessary to produce mammals genetically and biologically uniform is therefore too great for the patience and resources of most investigators.

As before stated the wild house mouse has been used in this experiment. If collected in the same locality one can be sure of the stock for several reasons (1) mice very seldom migrate, they usually remain in the same building and no doubt sometimes breed in the same nest in which they were born. This is evidenced by the fact that slight variations in coat color tend to be restricted to the same corner of a building, etc. (2) The fact that relatively fewer adult males than females are present, thus necessitating close inbreeding. This inequality of the sexes is brought about possibly by conflicts between individuals of the male sex. Dr. Sewall Wright has pointed out that the fewer the males employed for breeding the quicker homozygosity in the strain is produced. An added precaution was taken in determining whether there is any difference in the two tumors. Both tumors were inoculated into the same mouse, the dBrA tumor into the right axilla, the dBrB tumor into the left. By this method we have eliminated the possibility of even a slight variation in the race employed by comparing the two tumors in the same soil. If there is any difference in the tumor cell we should be able to determine it, since the soil in each case is identical.

Beginning with the second week after inoculation, weekly observations were made by means of palpation. If there were any indications of the tumor, the relative size of the mass was charted, as accurately as possible, on coördinate paper. Six observations were made for each inoculation; it having been shown by previous experiments that if any indication of growth was going to develop it would appear in that period.

Not one mouse in a total of 160 employed for this report ever grew either tumor progressively for the entire period although several indications appeared as shown in the chart (Fig. 1).



Percentage Indications.
Weeks Observed.
FIG. 1.

It will be noted that the dBrB tumor gave the greater percentage indications throughout the experiment.

According to the binomial theorem, the possibility of all six points of the one curve being above the corresponding six points of the other is as 63 : 1, thus indicating that the two curves are significantly different, any odds above 27 : 1 being mathematically significant. There is a mathematically significant difference between the per cent. of indications of the two tumors when the results are massed, this being 8.6 times its probable error. The analysis of the observations by successive weeks shows that the first three week periods differ significantly in the two tumors, the difference in each case being more than three times its probable error. The slight rise in the curves in the last two periods is not real but due to the dying off of some negative individuals during an epidemic. The last three points approach mathematical significance and possibly will be significantly different when more numbers are obtained. Since both curves are approaching zero there would be convergence. For that reason the difference between corresponding points would not be as great as between points at the other end of the curve.

We are, therefore, led to the conclusion that the two histologically identical tumors possess different physiological reactions. Wherein lies this difference? This cannot be explained by fluctuations in the tumor cell. Within insignificant variation limits, the two tumors have retained their own reaction potentiality throughout the whole experiment.

Physiological differences of tumors of the same general type may, therefore, be independent of *histological* differences. There are two remaining explanations, either (*a*) cytological or (*b*) genetic. The cytological explanation involves fluctuations from the normal type of mitosis, amitosis, etc. Such explanations are not wholly acceptable to the investigators who approach the tumor problem from another angle.

We are led to the conclusion that it is in the genetic constitution of the individual that we are to look for the underlying causes that undoubtedly determine susceptibility to transplantable tumor tissue.

It has long been recognized that tumor cells are not distinct from normal cells. They only differ in their ability to grow indefinitely. Since normal tissues are, to a large degree, dependent upon the genetic complex of the individual, may we not also look for the causes underlying susceptibility to transplantable tumor as being similarly correlated with genetic factors?

We are indebted to Drs. James Ewing and H. J. Bagg of the Memorial Hospital, New York City, for their kindness in analyzing the material histologically.

28 (1610)

On certain poisonous substances produced in bacterial cultures.

By **HANS ZINSSER, JULIA T. PARKER** and **ANN KUTTNER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, New York City.*]

In a paper published by one of us last year we called attention to the fact that it was quite likely that not all the toxic substances produced in cultures by bacteria can be peremptorily classified as either exotoxins or endotoxins. The writers working with a number of different organisms, biologically unrelated, have found poisonous substances of moderate potency developed both on fluid and on solid media which they believe should not be regarded at the present time either as specific or antigenic exotoxins, or as endotoxins. Indeed, it seems quite impossible at the present time to definitely classify these substances, for which reason they are referred to in our laboratory, for the present, as the "X" substances.

I.

Our first observations on this substance were made with Miss Kuttner on hemolytic streptococci. It was found that supernatent fluids or filtrates of such cultures grown for 20 to 22 hours either aërobically, or better, with partial anaërobiosis, regularly produced sickness in rabbits, although there was great irregularity in potency. Potency was never great. Rabbits intravenously injected with such culture fluids always showed an incubation period of from 60 to 90 minutes, at the end of which time a regular train of symptoms ensued, consisting of respiratory difficulty, weakness, refusal to eat, flattening out on the bottom of the cages, muscular relaxation, half closure and often watering of the eyes, in which condition they either remained for 2 to 3 hours, and then recovered, or in some cases died acutely in anywhere from 2 to 3 hours to two days. The dosage necessary for this ranged between 3 and 6 cubic centimeters. It was apparently impossible to control the potency of these substances. We have not found out the reason why, in some cases we obtained very severe symptoms and death, but in most instances only moderate, but distinct illness.

Experiment has shown that the poisons are not extractives of the bacterial bodies.

They are weakened and sometimes destroyed by heating to 80° for 30 minutes.

They seem to be relatively more potent for rabbits than for guinea pigs.

They cause a marked reduction of leucocytes and probably in this sense have a definite agressin-like action. Small doses of streptococci injected with such substances killed, in some instances, in a shorter time than did many times the amount of washed organisms of the same cultures.

In four separate experiments it has seemed distinctly as though the first isolation of certain streptococci from the human body gave rise to more potent poisons than we were ever able to produce with the same organisms in later generations.

Time experiments seem to show that the poisonous substance is more plentiful in the cultures at about the period of the highest growth energy of the organisms.

There is no indication that there is any relationship between these substances and virulence.

The substances were produced on simple hormone media with almost the same potency with which they appeared on the richer protein media such as chocolate broth. In general, however, the potency was in favor of the richer media.

Many of the more accurate determinations of biological properties, such as certainty about the temperature of destruction, deterioration at room temperature, and relative toxicity for various animals, are difficult to determine with absolute certainty because of the great fluctuation in potency of the substances obtained and the probable differences in susceptibility of individual rabbits.

In spite of fluctuations of potency, however, some degree of toxic action was never absent and its importance in connection with animal immunization necessitated further study.

As to the formation of these substances in the body of the animal, we can say, at the present time, only that the symptoms produced after an incubation of from 1 to 1½ hours in a rabbit are similar in general to those which appear in a rabbit at the time when the streptococci begin to appear in the blood stream in considerable numbers.

II.

A very important consideration is whether these substances represent toxic split products of the culture fluids produced by the bacterial growth, rather than toxic products secreted by the bacteria. In favor of regarding them as split products of the medium is the fact that they were more potent when the media was rich in proteins. Also suggesting this is their apparent non-specificity, in that, as will be shown directly, we obtained similar substances from many other organisms including non-pathogens. Again, in our experiments up to date, as will be seen, we have no reason to believe that they are in any sense antigenic.

Against the conception that they are split products of the constituents of the media are the following facts:

The substances were often high in potency from simple hormone media, as well as from media containing rabbit's blood and other proteins.

It seems that they are more potent in young cultures at the height of their growth energy. Potency seems to be at its maximum after 22 hours, decreasing with 48 and 72 hour incubation periods.

There is definite diminution of potency by filtration.

That there is a definite loss of potency by heating it 80° or below.

That there is a definite incubation time, rarely shorter than one hour, and never shorter than 40 minutes, even when large doses of relatively high potency are injected.

The incubation period which is characteristic of these "X" substances, differentiates them sharply from histamin and tyramin. Furthermore, the symptoms produced in rabbits by injections of ergamine differed distinctly from the symptoms produced by the "X" substances. The histamin when injected into rabbits in minimum lethal doses produced immediate acute death, and when injected in sublethal doses produced immediate sickness, followed by recovery, the animal usually being perfectly well after ½ hour. The supposition that the toxic action of the "X" substances might be due to substances analogous to histamin produced by the growth of the bacteria in the culture media, is thus rendered unlikely since the symptoms produced by the latter class of substances are immediate. The relatively greater susceptibility of rabbits to the "X" substances as compared to that of guinea pigs also excludes substances of the histamin class, since no such difference has been noted in experiment with histamin. Also histamin and other toxic amines when produced in cultures of bacteria are usually found in cultures some days older than those used by us. Chemical and pharmacological analysis of our poisons has not yet been undertaken.

III.

In order, further, to investigate the importance of split products of the medium, we attempted to produce the poisons with cultures grown on solid media. When the streptococci were grown in flat bottles on agar, chocolate agar, or Loeffler's medium for 20 or 22 hours, then washed up in salt solution, shaken for 2 minutes, filtered through Berkfeldt, and the filtrates injected intravenously into rabbits, symptoms exactly analogous to those produced by liquid culture products were observed, with the constant and definite incubation time and the same train of symptoms. In general, the solid media washing substances were less potent than the broth culture filtrates, but there was great irregularity and occasionally these filtrates killed acutely.

In all cases proper controls were made in order to eliminate agar-anaphylatoxin injury, a factor which was insufficiently controlled in similar experiments of Kraus, Kraus and Doerr, and Arima. This factor too is totally eliminated in those of our experiments in which we grew the bacteria on Loeffler's medium.

IV.

The symptoms produced in the animals treated with streptococcus preparations were entirely analogous, though less severe, as a rule, to those observed in parallel experiments carried out with the influenza bacillus in our laboratory by Mrs. Parker. In consequence, with Mrs. Parker and Miss Kuttner, we undertook a comparative study of the production of similar poisons with streptococci, typhoid bacilli, and influenza bacilli, and did a few isolated experiments with dysentery, meningococcus, pneumococcus and staphylococcus.

With Gram-negative bacilli like typhoid, we felt that we had to be particularly careful to eliminate the extractive substances spoken of as endotoxins. In consequence, we grew typhoid bacilli on various media for the shortest possible periods, consistent with good growth using filtrates of 4, 6, and 12 hour cultures. These always gave definite sickness in rabbits intravenously injected, when 4 or more cubic centimeters were administered, and in all cases the incubation time and the symptoms were in every way similar to those produced by streptoccus and influenza filtrates. Comparison of such young 6 hour culture filtrates with the filtrates of 6 and 10 day cultures showed that the 6 hour filtrates made the rabbits quite as sick within the first 2 or 3 hours as did the extraction posions from the old cultures. The symptoms were somewhat different, however, rabbits receiving those from young cultures usually recovering in a short time, whereas, the others went on to a typical endotoxin death.

We may say in passing that we do not believe it possible even with 6-hour growths to eliminate the presence of extractive substances because rabbits treated with 6-hour filtrates always developed agglutinins and complement-fixing bodies, a fact which shows that investigators who have worked with filtrates of 5- or 6-day cultures of typhoid and dysentery could never have worked with pure exotoxins. And the sera produced with such 5-day filtrates must have contained considerable amounts of sensitizer to the bacterial protein to which some, at least, of their always limited protective action must be attributed. This is rendered still more likely by the fact that such sera have been strongly protective only after incubation with the antigen in vitro before injection. In general, as far as we have gone, it has seemed that the filtrates of young typhoid cultures were very much more toxic for rabbits than for guinea pigs, being, in this, similar to streptococcus and influenza filtrates. Filtrates of old cultures on the other hand were relatively more toxic for guinea pigs.

As to deterioration of the 6-hour typhoid substances at room temperature, our results have been irregular, except that it has seemed that after 24 hours' standing, there was distinct loss of toxicity in isolated instances.

In one experiment there was marked deterioration of toxicity after heating to 70° for 30 minutes, and in 3 other experiments heating to from 75° to 80° for 30 minutes almost completely destroyed the toxicity of 6 and 12 hour typhoid filtrates.

There was, thus, marked similarity between the influenza and the typhoid substances, in all these particulars.

With both of these organisms experiments were now done with growths on solid media.

Cultures on various solid media were allowed to grow 6 hours in the case of the typhoid bacilli, in order to prevent as far as possible any bacterial cell death. Filtrates from such washings regularly produced sickness analogous to that described above.

Although acute death was sometimes obtained, in general the filtrates of washings of typhoid cultures as young as these have not killed, producing only marked illness after the regular incubation time.

With the influenza bacillus it was necessary, because of the nature of the organism to grow the cultures on chocolate agar. The shortest period of growth of these organisms on solid media was 12 hours.

Isolated experiments were done with other bacteria. Toxic filtrates were obtained from a 23-hour meningococcus culture on horse-serum-hormone-broth; from a 5½-hour culture of *prodigiosus* on rabbit-serum-hormone-broth, and mild reactions were obtained with a similarly prepared 23-hour Shiga bacillus culture filtrate.

With solid media washings, we produced definite sickness after the ordinary incubation time, and death in 18 hours with a filtrate from the washings of a 20-hour *prodigiosus* culture on hormone agar, and 2 deaths (in 2 and 3½ hours, respectively) with filtered washings of 5½ hour *B. coli* cultures on similar media. Mild but definite illness followed in two cases after the injection of filtered washings of 5½-hour cultures of the Flexner bacillus on hormone agar.

In a few isolated experiments we failed to obtain any indications of the formation of the poisonous substances with staphylococcus and pneumococcus, Type I.

All attempts to establish specificity or non-specificity in immunological experiment by treating rabbits with filtrates of the various organisms and testing cross protection, have failed because, so far, we have no evidence of antigenic action of these substances. Repeated injection of rabbits with doses too small to produce definite sickness that is, doses of 1 to 2 c.c., have resulted in emaciation, gradual falling out of hair, and eventual death of the animals by apparent chronic injury. In such experiments rabbit protein was used in the media to exclude anaphylactic injury. We have not yet been able to obtain any of these poisons in sufficient potency to permit us to draw conclusions from systematic experiments concerning their action after intraperitoneal and subcutaneous injection. Although we have obtained some indication of injury after subcutaneous injection this point calls for further study.

SUMMARY AND CONCLUSIONS.

It will be noticed that whatever the organisms used, whatever the medium, and whether the substances used were filtrates of liquid cultures or filtrates of washings of young cultures grown on solid media, the general nature of the toxic effects, incubation time, etc., were always the same. Autopsy findings in all such cases in which acute death was obtained, never showed lesions that might in any way be regarded as characteristically defining the pharmacology of the poisons. These facts, together with our failure to establish specificity by any immunological tests, inclines us to believe that in all cases the poisons were of a similar, perhaps of the same nature.

The fact that there was a regular incubation time, that the toxic substances seemed to show heat instability, that they had a relatively greater toxicity for rabbits than for guinea pigs, and that they appeared in young cultures with apparent diminution as the cultures grew older, constitute evidence which prevents our dismissing them, peremptorily as split products of constituents of the media.

We cannot, at the present time, characterize them definitely, but we are safe in asserting that they are neither exotoxins in the ordinary sense, and cannot be regarded as endotoxins or extractive substances from the bacterial bodies.

We believe that it is these substances which have been regarded by many writers as exotoxins of streptococci. Also, it is not unlikely that they represent the substances which have been regarded as specific exotoxins by Kraus and his co-workers, and by Arima, who produced toxic effects in rabbits by the injection of the washings of typhoid and dysentery cultures grown on solid media. We have recently been told by Dr. Anderson also that in immunizing horses with agar cultures of meningococci, he has found it advisable to wash the cultures once in salt solution in order to diminish the toxic effects noticed in horses when this was omitted. Such observations also would be explained by a knowledge of the substances we have described. We may state that recently a horse turned over to Mrs. Parker. by Dr. Park for immunizing with influenza culture filtrates, died after the nineteenth subcutaneous injection. These cultures were grown on horse-blood-chocolate broth, a fact which should exclude ordinary anaphylactic effects.

We have worked with these substances for considerably over a year, making many cultures in many different ways and using several hundred rabbits, and have found that there are great experimental difficulties which make it impossible for the present to define the biological properties of these substances with the accuracy which can be applied to more potent substances such as the true toxins. We have no experimental evidence to show that these "X" substances are produced by streptococci, or other bacteria, during their growth within the animal body. The condition of rabbits injected with cultures at a time when general septicemia·ensues some hours before death, is strikingly similar to that which is produced one or two hours after injection with the filtrates, and the filtrates produce a leukopænia similar to that which occurs as the body is being overwhelmed by the streptococcus infection. However, the low potency of the poisons and other difficulties of working with them, makes it impossible at the present time to approach this problem more closely.

We cannot, therefore, at the present time do anything more than submit the observed facts with the hope that experimental difficulties may be overcome in the future.

Such as they are, however, these "X" substances are very definite and probably non-specific products which appear early in cultures and with regularity, and which must be taken into consideration in all work in which bacterial cultures or their derivatives are injected into animals.

29 (1611)

Observations on anaphylaxis in lower monkeys.

By **HANS ZINSSER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

It is always dangerous to apply to any species of animal reasoning or theories deduced from experimental observations upon another species. In no phase of immunological work is such deduction more unjustified than in anaphylaxis where we know that the reactions induced in different species by a reinjection of proteins vary from each other in fundamental, physiological mechanism. It is of course unlikely, therefore, that we can justly draw conclusions from monkey experiments to conditions prevailing in human beings. But some of those who have regarded the occurrence of true anaphylaxis in the human being as at least doubtful, have, at the same time, cited the difficulty of producing anaphylactic reactions in monkeys in analogy. The problem is hardly one warranting a great deal of extensive research, but in connection with other work going on in our laboratory, we have found it important to investigate, for ourselves, the true conditions prevailing in the lower monkeys.

The production of antibodies in monkeys has for some time been a matter of controversy. Uhlenhuth[1] injected human serum into *Macacus rhesus* and found that specific precipitins were formed. Berkeley,[2] in 1913, reinvestigated this question on *Macacus rhesus* and on a Java monkey, and found that these animals treated with human, horse or dog sera, receiving four injections of 1 to 2 c.c. of these sera, produced neither precipitins nor complement fixing antibodies for the antigens used. He does not believe, therefore, that it would be possible to utilize antisera from lower monkeys for the forensic differentiation of human and monkey sera, as suggested by Uhlenhuth.

There has not been a great deal of systematic work published upon monkey anaphylaxis. Yamanouchi[3] was unable to produce

[1] Uhlenhuth, Kolle and Wassermann Handbuch, Second Edition, 1913, iii, 264.

[2] Berkeley, Univ. of Calif. Publications in Pathology, 1913, ii, 105.

[3] Yamanouchi, *Compt. Rend. de la Soc. Biol.*, 1910, lxii, 1,000.

active anaphylaxis in the lower monkeys against horse serum, and found that the serum of the lower monkeys did not sensitize guinea pigs passively. His horse serum injections produced no effects in the monkeys, but in his report there is no analysis of antibody production or other details which would permit one to draw one's own deductions.

As a necessary accompaniment to some other work we were engaged in, we thought it desirable to do a few experiments, worked out in careful detail, on the problem of antibody production and anaphylaxis in the lower monkeys, and we used, for this purpose, two *Macacus rhesus*, 1 *Macacus cynomolgus*, and 2 South American ringtail monkeys which happened to be available in the laboratory at different times.

EXPERIMENT I.

The first experiment was carried out in order to determine by the most delicate test available to us, namely, the isolated uterus method of Dale, whether or not a single injection of horse serum would produce any degree of hypersensitiveness in a monkey, analogous to that produced by such injections in guinea pigs.

For this purpose this monkey, *Macacus cynomolgus*, was etherized on March 13, 1920, and the right fallopian tube removed, and mounted in the Dale apparatus, in a 200 c.c. Ringer solution bath. The monkey was sewed up aseptically, and given 3 c.c. of sterile horse serum intravenously after the operation.

After the fallopian tube had begun to show regular rhythmic contractions, as shown in the chart, 1 c.c. of horse serum, diluted in 10 c.c. of Ringer's, was introduced into the bath, and this process was repeated twice, at about five-minute intervals, in order to make sure of the quantities of horse serum which could be given without eliciting reaction in the uterus of the untreated or normal monkey.

In the interval between March 13 and April 27, intracutaneous skin reactions with 0.2 c.c. of a 1–15 horse serum dilution were done on March 22, March 30. April 14, and April 22, and titrations of monkey serum were made on the same days, against horse serum, and against anti-horse serum, to determine the formation of antibodies, or the persistence of antigen, respectively. In no case was a definite skin reaction obtained, and in no instance was any precipitating antibody discovered. On April 27, the monkey was again etherized and the other fallopian tube removed by operation. The monkey was again sewed up and the removed tube placed in the Dale apparatus as before. The same horse serum instillations were made, again without the slightest effect, as shown by the attached records (see Fig. 1).

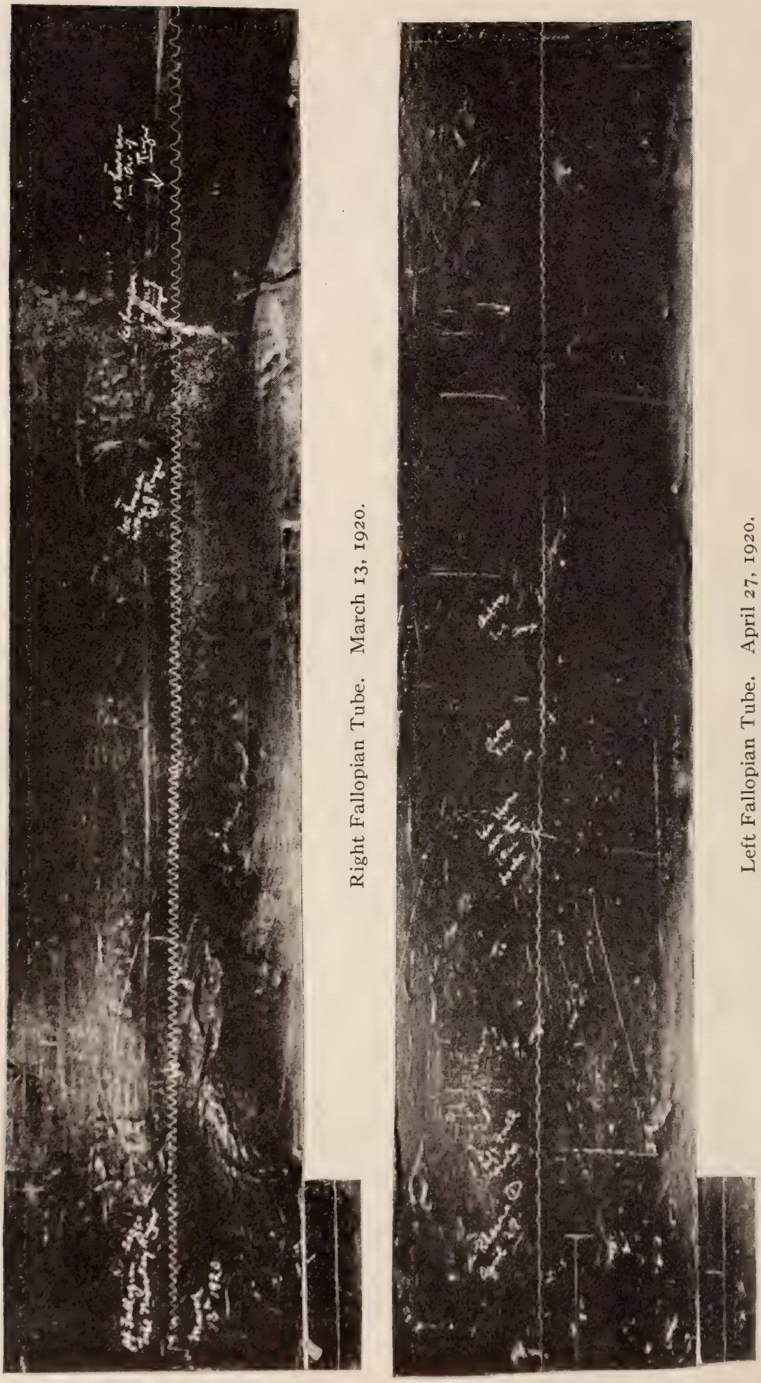
At the same time, the monkey, as soon as he came out of the ether, was reinjected with 3 c.c. of horse serum, intravenously, without showing the slightest reaction that could be interpreted as anaphylaxis, either immediately or later.

This experiment shows definitely that a single injection of horse serum did not elicit antibody formation or anaphylactic symptoms in a *Macacus Cynomolgus.*

EXPERIMENT II

Monkey II, Small Cebus Monkey.—On March 15 received 4 c.c. horse serum intravenously. Skin reaction 0.1 c.c. of 1–5 dilution of horse serum, intracutaneously, done just before injection, was entirely negative. *March 22, skin reaction, negative, 2 c.c. horse serum* intravenously, no symptoms, except slight shivering, two soft defecations, tremor in the legs beginning 12 minutes after injection; perfectly well in 30 minutes. April 7, titration of serum against horse serum shows a faint but distinct ring in the tubes containing the horse serum, dilution of 1–2, and 1–10. In consequence, on April 8, a skin test was done with 0.05 c.c. of 1–10 horse serum, which showed slight redness coming on within 3 minutes, surrounded by a small edematous area, which faded out within 30 minutes, and was regarded by several observers as distinctly different from other reactions produced on the monkeys. This was interpreted as a mildly positive reaction.

Immediately after the skin reaction, 1.5 c.c. horse serum was intravenously injected. After the injection, seems uncomfortable and abnormally quiet. After 30 minutes, marked redness of the face, and the lower part of the face and lips are distinctly swollen and edematous. This was noticed by several observers, and checked up by our technician who handles these monkeys continuously. This lasted about 2 hours, when it gradually returned to normal.



Right Fallopian Tube. March 13, 1920.

Left Fallopian Tube. April 27, 1920.

Fig. 1.

On April 22 this animal was again titrated against horse serum, and distinctly positive ring tests were obtained against horse serum dilutions of 1–5, 1–10, extremely faint and hardly noticeable in 1–20.

May 5, again titrated with practically the same result, except that reactions were somewhat weaker than on April 22. Skin reaction done on this day shows a doubtful reaction which, however, might have been regarded as a faintly positive one. On this day, 4 c.c. horse serum injected intravenously was followed immediately by shivering, watering of the eyes, and monkey continuously rubs nose and eyes as though they itched. No other symptoms.

In these monkeys, in a considerable number of titrations, the monkey serum titrated against anti-horse rabbit serum showed reactions which seemed positive again and again for sometimes as long as two weeks after the last injection, with appreciable diminution. This made us suspicious of the specificity of the reactions, and we obtained, through the courtesy of Dr. Cecil, specimens of the serum of 7 normal monkeys which we titrated against 4 different specimens of antihorse serum from 4 different rabbits, and found that in almost all cases precipitates were given by antihorse serum against the normal monkey sera in dilutions as high as 1–10. These monkeys all belonged to the Macacus variety, and it was obvious that our apparent antigen persistence was due to a normal reaction between anti horse serum and normal monkey serum. Similar titrations done at the same time against five different normal rabbit sera give only one ± reaction between normal rabbit "5" and normal monkey "5." All other reactions were entirely negative.

Although we cannot explain this, it seemed possible that we might have been dealing with a peculiarity due to unsuspected antigenic relationship between monkey serum and horse serum, a fact which might also possibly explain the failure of the monkeys to react to the horse antigen.

In consequence we shifted further experiments to egg white, instead of horse serum.

Three monkeys were systematically treated with egg white made up in concentrations of 1–5, by slaking egg white in salt solution and filtering through moist paper. Before these monkeys were injected, their blood serum was titrated to ascertain whether there were non-specific cross reactions between monkey serum and egg white, and no such reactions were obtained. Skin reactions and titrations were done at frequent intervals in the course of the experiments, and were we to detail all of these, we would make a long communication of a relatively simple matter, since there are no technical difficulties in the methods used, sufficiently familiar to all serologists. We will, therefore, omit protocols and state the pertinent results briefly.

Monkey III, Macacus Rhesus.—Preliminary titration showed no reactions with egg white or with anti-egg rabbit serum. An attempt was made to repeat the fallopian tube experiment with this monkey, but owing to the relatively much deeper pelvis of the *rhesus* variety and the astonishingly small fallopian tube of this young female, the results, although negative, were not convincing and will not be cited.

May 24, 2 c.c., 1–5 egg white, intravenously. Intracutaneous skin reaction before this injection, negative.

June 1, no antibodies to egg white.

June 17, skin reaction negative, intravenous *injection of 10 c.c., 1–5 egg white,* no reaction.

July 7, skin reaction negative; titration shows a very faint positive precipitin reaction against egg albumin, 1–10, and anti-egg rabbit serum shows very faint reactions up to dilutions of monkey serum of 1–5.

July 12, 10 c.c., 1–5 egg white, intravenously. Immediate moderate respiratory distress; irregular and rapid breathing within 2 minutes; staggers and turns about as though loss of equilibrium; hind legs weak; holds onto cage; falls down twice, but immediately up again; seems much distressed, but is practically well again in 5 minutes. All of these symptoms took place after monkey had been left alone in cage and effects of handling could be excluded.

July 27, titration, definite and fairly strong precipitin reaction against egg white in dilution of 1–10. Anti-egg rabbit serum shows no antigen in the monkey serum. Skin reaction on this day, negative; 10 c.c. of 1–5 egg white injected, intravenously; after 5 minutes rapid respiration, great pallor, falls to the ground two or three times, but immediately gets up again and keeps himself upright by holding onto the cage. Very distinctly in distress; gradual recovery within 10 to 15 minutes.

August 13, skin reaction negative, 7 c.c., 1–5 egg white intravenously injected; after 3 minutes is distinctly distressed, respiration very fast; face suddenly very red; legs weak; falls to the ground, but is immediately up again, clinging to the cage; returns to normal after 10 minutes.

Monkey IV, Macacus Rhesus.—Young female mate to preceding monkey. This monkey was treated exactly like the preceding. Titrations, skin reactions and injections were run parallel to Monkey III. At no time did we get any indication of positive skin reaction, nor did we, in four separate titrations carried out between May 24 and August 13, ever find the slightest trace of antibody formation. We never obtained, after injection of egg white, reactions comparable to those obtained in Monkey III, nor anything else that could be interpreted as anaphylactic shock.

Monkey V, Brown Ringtail Monkey.—The intention was to prepare this monkey by a number of injections before tests were done on him.

October 15, 10 c.c., 1–5 egg white, intravenously
October 18, " " " " "
October 20, 6 " " " " "

October 26, bled and titrated; no antibody; slight trace of antigen in dilutions up to 1–10 of the monkey serum.

October 30, bled and titrated with all necessary controls; no antibody and no antigen in monkey serum, although readings were made up to as long as 48 hours in the ice-chest.

November 5, skin reaction negative. Titration shows no antibody and no antigen; 14 c.c., 1–5 egg white injected, intravenously. Within a few minutes shivering and a general tremor of body and limbs; monkey lies down and is weak, very apparently sick; will not eat, and seems to prefer lying on his side when left alone. There are none of the ordinary signs associated in our minds with anaphylaxis, but for 4 to 5 hours after the injection, the monkey allows himself to be handled without much protest, remains lying on his side, and acts generally weak and sick; at the end of 5 hours, he gradually begins to improve, and eats a little bread, and from that time gradually recovers.

Since, in this monkey, as well as in the preceding ones, we were never able to obtain any considerable amount of antibody, and failed to find antigen in the blood within relatively short periods after injection, we naturally wondered what became of the egg white injected. For this reason we placed this last monkey into a metabolism cage after the last injection, and within 2 hours after the injection of 14 c.c., 1–5 egg white, we obtained through a filter-covered funnel, about 12 c.c. of clear urine. This urine was light colored, and neutral to litmus, and was titrated against anti-egg rabbit serum in various dilutions. It gave very heavy and rapidly appearing precipitin reactions against anti-egg rabbit serum in dilutions ranging from concentration to 1–10, and distinctly and visible reactions in dilutions as high as 1–40. Parallel titrations against anti-ox and anti-streptococcus rabbit serum, carried out to make sure that we were not dealing with any fortuitous property of the urine which would cause reactions with rabbit serum in general, were entirely negative.

CONCLUSIONS.

In the preceding monkey experiments we have confirmed the observations of a number of workers, especially of Berkeley, that monkeys are very poor animals to use for antibody production and, incidentally, observed that antihorse rabbit serum often gives definite reactions with normal monkey serum.

In the first experiment with a *Macacus cynomolgus*, carried out by the separate testing of the two fallopian tubes by the Dale method, it appeared that a single injection of horse serum produced not the slightest trace of an anaphylactic sensitization. This was borne out by the results of intravenous injections into the same monkey. No antibodies were formed in this monkey as far as could be ascertained by the precipitin test.

In Monkey II, a small *Cebus* monkey, and Monkey III, a young female *Macacus rhesus*, small amounts of antibody were formed after repeated injection, and moderate reactions, probably of an anaphylactic nature, were obtained.

In Monkey II, 17 days after the second injection of horse serum, a small amount of antibody was found in the serum, and reinjection elicited a reaction which, it seemed to us, as justifiable to regard as mildly analogous to human serum sickness, with definite redness and swelling, and some edema of the lower part of the face and lips, lasting about 2 hours. On May 5, 27 days after the third injection, again with small amounts of antibody present in the serum, this same monkey showed nothing but watering of the eyes, and extensive itching of the nose and eyes, with shivering and, otherwise, reactions that could be regarded as slight anaphylactic symptoms if they were observed in human beings.

In Monkey III, treated with egg white, 25 days after the second injection, with slight amounts of antibody present in the serum, a definite reaction was obtained by reinjection of 10 c.c., 1–5 egg white intravenously, described in the text under this monkey, unmistakably as a severe reaction of some kind, coming on within 2 minutes, and completely over in 5 minutes. This same monkey 15 days after the third injection, and 17 days after the fourth injection, always with a small amount of antibody in his serum, manifested similar reactions on each occasion. In Monkey IV and Monkey V, antibodies were never found and no symptoms that could, in any way, be spoken of as anaphylactic, either in nature or in time of occurrence, were observed.

We believe that we are justified in concluding that anaphylaxis is very difficult to obtain in the lower monkeys, probably cannot be obtained by a single preparatory injection, but that occasionally definite mild anaphylactic reactions can be obtained in these animals, in one case simulating some of the symptoms associated in human beings with serum sickness (Monkey II), and in our series this occurred only in monkeys in which small amounts of antibody could be demonstrated.

Why antigen should disappear with relative speed, and no antibodies appear in some of these monkeys, may possibly be explained by the fact that in the last monkey, we observed that, within a few hours, the egg white had passed through the kidney into the urine with remarkable speed.

To some extent our results with the monkeys indicate analogy to conditions in human beings as found by McKensie in his studies upon the parallelism between the antibody curve and serum sickness, but at the same time they show that human beings are considerably better anbitody producers than are the monkeys, and if the parallelism between the two conditions is as we surmise, one would expect the greater severity of symptoms which occurs in human beings.

30 (1612)

Dissection and injection studies on the Amœba.

By Robert Chambers.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

The species used was *Amœba proteus.* By means of a micropipette liquids of various kinds were injected and the effect noted.

Oils form spherical droplets which are carried about in the cytoplasmic currents. A large drop is usually expelled. Immediately on being extruded the drop tends to flow over the surface of the *Amœba* thus partially engulfing it.

Distilled or spring water diffuses through the granular endosarc diluting it. The dilution is followed by a contraction of the endosarc and the massing of a hyaline fluid between the endosarc and the external pellicle of the *Amœba.* This dilates the area usually termed the ectosarc. The fluid soon accumulates on one side of the *Amœba* in the form of a blister which is ultimately pinched off.

A number of acid indicators were injected. The color reactions showed that the protoplasm of the *Amœba* is more acid than its environment. Upon death the colors change to those characteristic of the surrounding medium.

The difference in behavior of living protoplasm to "basic" and to "acid" dyes is striking. The "basic" dyes used were all chlorides of colored basic radicles and the "acid" dyes, potassium or sodium salts of colored acid radicles. In every case the "basic" dyes had a coagulating and the "acid" dyes, a liquefying effect on the protoplasm.

In the case of the "acid" dyes, when the effect is local, the healthy non-colored portion of the endosarc shrinks away from the colored liquefied area. This liquid accumulates under the pellicle in the form of a blister and is ultimately pinched off.

If the "basic" dye be relatively nontoxic its injection results in a coagulated area which is localized as a colored lump of inert material. This lump is carried about in the protoplasmic currents. The color gradually diffuses out of the lump and stains many of the cytoplasmic inclusions in the *Amœba.*

Dissection indicates that the granular endosarc is capable of easily reverting from a fluid to a solid state and vice versa. Peripheral to the endosarc is a hyaline liquid zone, the ectosarc, which is bounded externally by a very thin, extensible, pellicle. The extosarc can be enlarged by a hyaline liquid extruded from the endosarc.

In the formation of a pseudopod a localized area of the pellicle softens. The accumulation of liquid in the ectosarc immediately under this area produces a bulge. The more jellied endosarc at the base of the bulge liquefies and a liquid suspension of granules streams into the bulge and up to its tip where it spreads out and flows back peripherally in the manner of a fountain flow. The granules heap up around the base of the bulge where, by means of a jellying process, a semisolid wall is built about a central liquid channel. Retraction of a pseudopod is accompanied by a reversal of the jellied to a liquid state.

An undisturbed *Amœba* usually forms numerous pseudopodia. Upon continued agitation a broadly lobate pseudopod is formed. The jellying process of the backward flowing endosarc is diminished. The base of the pseudopod, consequently, broadens more and more until all of the endosarc reverts to a liquid state and the entire body of the *Amœba* becomes transformed into what one may term a single pseudopodium within which vortical currents occur analogous to those of a chloroform drop creeping along a bed of shellac under water.

The motile activities of an *Amœba* depend upon a delicate balance between the liquefying and solidifying tendencies of its protoplasm. The most recently solidified regions are the ones that most readily liquefy. In this way a gradient exists with a definite antero-posterior axis. The posterior end consists of a heaped up mass of jellied material which is more resistant than other parts to the liquefying process necessary for the formation of pseudopodia. In an actively moving *Amœba* the amount of such material is very small and pseudopodia may form on either side thus tending to mask its presence. Exceptionally the posterior end may be made to liquefy but usually the inert posterior end compels an *Amœba*, in order to retrace its path, to turn about.

31 (1613)

Concerning the antiseptic action of some aromatic fumes.

By **David I. Macht** and **William M. Kunkel.**

[*From the Pharmacological Laboratory, Johns Hopkins University.*]

The recent World War was instrumental, in connection with the impetus given to the search for antiseptics and parasiticides, in calling attention to the powerful antiseptics and germicidal properties of various essential oils; as for instance so well described by Cavel[1] and Fränkel[2]. This antiseptic action of volatile oils is undoubtedly responsible for the remarkable medicinal virtues of ancient balsams, especially in relation to the treatment and healing of wounds. An ethnological study of the habits and customs of ancient peoples, especially in the Orient, cannot fail to call attention to the extensive employment of incense, perfumes and fumigations among these people. Incense was burned in connection with the religious and sacrificial offerings on the one hand, and for esthetic purposes in private homes on the other. Again, powerfully odoriferous substances are in great vogue in the Orient as perfumes and not only are such drugs applied to clothing but very frequently the orientals fumigate their naked bodies directly with the smoke of aromatic herbs and spices, burned over glowing coals. These circumstances suggested to the authors the possibility that such perfumes and fumes may serve a hygienic as well as esthetic purpose by exerting an inhibitory effect on the growth and spread of microörganisms. Accordingly some experiments were undertaken in order to ascertain the value of such an hypothesis.

The authors subjected a number of gums, spices and other odoriferous substances to destructive dry distillation by heat and studied the effects of the fumes produced thereby on bacterial cultures. The following substances were investigated; gum olibanum, gum glabanum, storax, myrrh, saffron, cinnamon, benzoinum and various samples of domestic and Japanese incense. The various substances were heated in glass test tubes over a flame and the fumes were allowed to come in contact with various microörganisms, care being taken to exclude the germicidal effects of steam. The bacteria studied were: *B. coli*, and *B. pyocyaneus aureus.* In one series of experiments smears were made with live organisms on agar slants; then a given fume was poured into or over the test tube and the culture incubated. In another series of experiments agar or gelatin plates were inoculated with the bacteria and allowed to remain open under a glass bell jar, in which incense was burned. In still another series of experiments the various spices in powdered form, individually or in combination with each other, were incorporated in culture media and then an attempt was made to grow bacteria on those media. Control experiments were made by burning filter paper (cellulose), raw cotton and various woods.

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[1] *Comp. rend.*, 1918, clxvi, 827.

[2] Theropmonatofte, June, 1915.

The results of the experiments showed that the fumes of most of the gum and spices studied, notably gum galbanum, gum olibanum, cinnamon and saffron prevented the growth of freshly transplanted bacteria, in other words, exerted a distinct *antiseptic* action. When however such fumes were poured over or brought in contact with fully grown or luxuriously developed cultures of *B. pyocyaneus* and especially of *B. coli* the *germicidal* effect was not so marked. This was possibly due to the poor penetration of the fumes and the volatile oils, etc., carried over by them, into the deeper layers of the cultures.

It was not surprising to note that fumes produced by destruction of various woods and also of cotton gave similar results. Pure cellulose however was not antiseptic in this respect.

Inoculation of bacteria into culture media impregnated with the various aromatics produced a distinct inhibitory effect on their growth.

The burning of various forms of incense, especially of Japanese origin produced a distinct antiseptic effect, that is, inhibited the growth of organisms freshly inoculated on plates, which were exposed to such fumes in partially closed chambers, for periods varying from ten to thirty minutes.

While the experiments above reported were of a crude character the results obtained were of so uniform a nature, that the authors are inclined to conclude that the fumes produced by the burning or destructive dry distillation of various gums, spices and other aromatic substances of a similar nature, certainly tend to exert an antiseptic action on the bacteria studied. This is of course of interest not only from the scientific point of view, but also to the historian, as offering a possible explanation for the extensive employment of incense in connection with sacrificial rites, etc.

32 (1614)

The vitamine content of honey and honeycomb.

By **Philip B. Hawk, Clarence A. Smith** and **Olaf Bergeim.**

[*From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia.*]

Dutcher[1] concluded from experiments on pigeons that honey contained a small but negligible amount of antineuritic vitamine. Faber[2] did not find honey to protect against scurvy in guinea pigs.

The present authors carried out feeding experiments on albino rats, to determine whether the growth promoting accessories fat-soluble A and water-soluble B were present in white clover honey or in a mixed strained honey, and whether these honeys protected guinea pigs against scurvy.

Rats fed a diet lacking water-soluble B when compared with rats fed the same diet except that half of the carbohydrate was replaced by an isodynamic equivalent of either of these honeys, showed in five weeks an average gain in weight of only five grams in favor of the honey-fed rats.

Similar experiments on the addition of the strained honey to diets deficient in fat-soluble A showed almost similar failure of growth. The addition of comb honey, however, brought about cessation of decline and distinct gains in weight.

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[1] Dutcher, R. A., *J. Biol. Chem.*, 1918, xxxvi, 551.

[2] Faber, H. K., *J. Biol. Chem.*, 1920, xliii, 113.

The addition of twenty per cent. of honey to the diets of guinea pigs did not prevent, or appreciably delay, the development of scurvy in these animals.

33 (1615)

A study of the serum of complement-deficient guinea pigs.

By **ARTHUR F. COCA.**

[*From the Laboratory of the New York Hospital.*]

H. D. Moore[1] has described a race of guinea pigs that are naturally deficient in complement: the deficiency is inherited. A number of these animals were obtained from Dr. F. A. Rich of the Vermont State Agricultural Experiment Station and the sera of four were separately examined as to the presence of the components of complement. The findings were identical in all of the sera.

Both the mid-piece and the end-piece of complement are present. There is lacking only the so-called "third-piece," which is the thermostable element of complement that is destroyed by cobra-venom and absorbed by yeast cells and bacteria.

By itself, the complement-deficient serum produces no hemolysis when used in a quantity 40 times that of the minimal completely heomlytic quantity of normal serum. When mixed with a small quantity of inactivated normal serum (guinea pig or human) the complement-deficient serum hemolyses in about three times the minimal hemolytic quantity of normal guinea pig's serum.

The third piece of complement is not identical with the lipoid cytozyme (thrombokinase), since the blood of the complement-deficient guinea pigs clots normally. The third piece of complement is not absorbed out of normal serum by six volumes of ether.

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[1] *Journal of Immunology*, 1919, iv, 425.

34 (1616)

The potassium content of normal and some pathological human bloods.

By **VICTOR C. MYERS** and **JAMES J. SHORT.**

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

Our interest in the potassium content of human blood was aroused some time ago by the observation of Smillie[1] that poisoning may result from the administration of potassium salts to nephritic patients, and later confirmed in experimental uranium nephritis.

Observations on the potassium content of serum and whole blood have been made in fifteen cases, including four normal subjects, several cases of nephritis with marked nitrogen retention and a few miscellaneous cases. The potassium estimation was carried out by the cobaltinitrite method of Drushel essentially as described by Myers[2] in 1909 for spinal fluid.

The results obtained for serum in the four normal cases were somewhat less than 20 mg. per 100 c.c. calculated as K, and for whole blood about 8 to 10 times this amount. In five cases of nephritis with marked nitrogen retention the figures for the serum varied from 10 to 19 mg., in one mild case the potassium was 28 mg. while in a case of double polycystic kidney 35 mg. were found. The potassium content of the whole blood in these cases ranged from 52 to 148 mg. with an average of 100 mg. per 100 c.c. In all of these cases there was an associated secondary anemia and the total solids were diminished. No significant variation was found in a case of pure hypertension or a case of diabetic coma. Figures obtained for whole blood in two cases of pernicious anemia were low owing to the diminution in the red cell content.

In general the potassium content of whole blood tends to vary directly with the red cell content and the percentage of total solids. The few observations reported on cases of nephritis with marked nitrogen retention do not appear to support the suggestion that possibly some of the symptoms of uremia are due to a potassium poisoning as a result of retention of this element. Serum appears preferable to plasma for potassium determinations since hemolysis seems to be much more readily prevented.

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[1] Smillie, *Arch. Int. Med.*, 1915, xvi, 330.

[2] Myers, *Jour. Biol. Chem.*, 1909, vi, 115.

35 (1617)

On the elimination of phenolsulphonephthalein in acute mercuric chloride intoxication.

By **WM. DEB. MAC NIDER.**

[*From the Laboratory of Pharmacology of the University of North Carolina.*]

In two recent publications [1,2] on the toxic effect of mercuric chloride in normal and in naturally nephropathic animals, observations have been made concerning the relationship between the development of an acid intoxication and the acute kidney injury. In these animals the poison was given in large doses, 15 mgs. per kilogram, which eliminated the study of the development of the intoxication in the early stages.

The following preliminary note has as its object a study of the early stages of the development of an acid intoxication from mercuric chloride and the relationship of such a disturbance to renal function as is shown by the elimination of phenolsulphonephthalein.

Fourteen normal dogs have been used in the study. The animals were kept in metabolism cages, fed on bread with a small amount of cooked meat and given 500 c.c. of water by stomach tube daily. The animals were catheterized once a day and the urine examined for albumin and glucose. Centrifugalized samples were examined for casts. The phenolsulphonephthalein test was conducted according to the technique of Rowntree and Geraghty. Blood urea determinations were made by the method of Marshall as modified by Van Slyke and Cullen. The reserve alkali of the blood (R.p.H.) was determined by the method of Marriott. After the commencement of the intoxication the urine was ex-

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[1] MacNider, Wm. deB., "A Study of Acute Mercuric Chloride Intoxication In The Dog with Special Reference To The Kidney Injury," *Jour. Exp. Med.*, Vol. XXVII, 519, 1918.

[2] MacNider, Wm. deB., "The Susceptibility of Naturally Nephropathic Animals to Acute Mercuric Chloride Intoxications," *Jour. Med. Research*, Vol. XXXIX, 461, 1919.

amined for the presence of mercury by the method of Elliott. Following two days of normal observations, the animals were given 4 mgs. per kilogram of mercuric chloride by stomach tube. Prior to administering the mercury the dogs were partially narcotized by a subcutaneous injection of 0.25 c.c. of a 4 per cent. solution of morphine sulphate per kilogram. Such a procedure prevents the vomiting of the mercury. After the introduction of the poison the animals were studied by the methods just outlined. At definite periods during the course of the intoxication, 6, 12 and 24 hours, the animals were killed without the use of an anesthetic and autopsied. The liver and kidney were studied histologically.

The course of two experiments that are representative of the condition of the fourteen animals in the very early stages of poisoning by mercuric chloride are outlined in Table I. At the commencement of each experiment normal observations are recorded for two days prior to the commencement of the intoxication. A study of this normal record shows the urine to be free from both albumin and casts. The total output of urine in a twenty-four hour period has varied with the fourteen animals from 281 c.c. to 576 c.c. The elimination of phenolsulphonephthalein in a two

TABLE I.

ELIMINATION OF PHENOLSULPHONEPHTHALEIN IN ACUTE MERCURIC CHLORIDE INTOXICATIONS

Number of Experiment.	Duration of Experiment.	Mercuric Chloride Mgs. per Kg.	Urine in 24 Hours.	Albumin and Casts.	Phthalein per Cent.	R. p. H.	Blood Urea Mgs. per 100 Cc.	Mercury in Urine.
10	Normal Observation	0	368 c.c.	0	56	8.4	18	0
	Normal Observation	0	478 c.c.	0	53	8.4	20	0
	6 hours Animal killed	4 mgs. per kg.	571 c.c.	Trace no Casts	68	8.1	12	0
13	Normal Observation	0	281 c.c.	0	72	8.3	22	0
	Normal Observation	0	310 c.c.	0	70	8.3	24	0
	12 hours	4 mgs. per kg.	365 c.c.	0	78	8.3	17	Trace
	24 hours; animal killed		491 c.c.	Trace	74	8.1	17	Trace

hour period has ranged from 53 to 84 per cent. Blood urea determinations have varied from 18 to 27 mgs. per 100 c.c. of blood. The alkali reserve determinations in all of the animals have only shown a variation between 8.3 to 8.4.

The animal of Experiment 10, six hours after receiving the mercury showed a distinct increase in the output of urine. The urine in this six hour interval amounted to 571 c.c., as opposed to the normal maximum output for a twenty-four hour period of 478 c.c. At this early period a trace of albumin appeared in the urine. Casts were not present. Associated with the development of the albuminuria the reserve alkali of the blood was reduced from the normal of 8.4 to 8.1. The elimination of phenolsulphonephthalein was increased from the normal maximum output of 56 per cent. to 68 per cent. The blood urea showed a decrease from the normal maximum of 20 mgs. per 100 c.c. of blood to 12 mgs. The urine did not show the presence of mercury.

The animal of Experiment 13, Table I, received the same amount of mercury per kilogram as the animal of Experiment 10. The duration of the experiment was twenty-four hours. The course of the intoxication is in general similar to that shown by the previously outlined experiment. Twelve hours after the use of the poison the output of urine gave an increase of 55 c.c. over the normal elimination for the preceding twenty-four hours. By the end of the first twenty-four hours of the poisoning the animal had formed 856 c.c. of urine as opposed to the maximum output for a twenty-four hour normal period of 310 c.c. At the end of the first twelve hours of the experiment the urine was free from albumin but contained a trace of mercury. The elimination of phenolsulphonephthalein had increased from 70 to 78 per cent. The reserve alkali of the blood remained unchanged. The blood urea was reduced from 22 mgs. per 100 c.c. to 17 mgs. At the end of twenty-four hours of the intoxication the urine contained a trace of albumin. The elimination of phenolsulphonephthalein was reduced from 78 per cent. to 74 per cent. The normal elimination of the dye was 72 per cent. The reserve alkali of the blood was reduced from the normal of 8.3 to 8.1. The blood urea remained unchanged, 17 mgs. per kilogram.

The pathology of the liver and the kindey in these animals early in the intoxication from 4 mgs. of mercuric chloride per kilogram presents the following points of interest. The liver is pale and the lobulations well marked. Histologically the periphery of the loblues show cells which are edematous and necrotic. As the center of the lobule is reached these changes become less marked. The destruction of liver tissue is extensive when the duration of the intoxication and the amount of the poison administered is taken into consideration. The kidneys have appeared normal. On section they drip blood freely. The glomeruli are prominent. Histologically both the vascular and epithelial elements of the kidney have failed to show evidence of degeneration. The capillaries of the glomeruli have been well filled with blood and have generally obliterated the subcapsular space. No exudate or hemorrhage into the subcapsular space has been observed. The tubular epithelium, especially that of the convoluted tubules, has appeared decreased in volume, giving undue prominence to the tubular lumen. The nuclei have appeared large in proportion to the cytoplasm of the cells and have stained intensely.

CONCLUSIONS.

1. When normal dogs are given mercuric chloride in the dose of 4 mgs. per kilogram there develops a well marked and constant diuresis. This mercury diuresis is not solely dependent upon the action of the mercury on the kidney during its elimination, for such a diuretic effect may be obtained when the urine is free from mercury.

2. In the early stages of an intoxication from such small quantities of mercury there is further evidence of its diuretic effect which is shown by an increase in the elimination of phenolsulphonephthalein.

3. Prior to, or associated with, the appearance of albumin alone, or albumin and casts in the urine, there occurs a reduction in the reserve alkali of the blood. This change in the acid-base equilibrium of the blood is not primarily a retention phenomenon dependent upon an injury to the kidney, for at the time of the reduction in the alkali reserve of the blood there is evidence of an increase in the functional capacity of the kidney, as is shown by an increase in the amount of urine formed and by an increase in

the ability of the kidney to eliminate phenolsulphonephthalein. Furthermore, at this stage of the mercury intoxication the kidney shows no evidence of injury but shows on the contrary a convoluted tubule epithelium with nuclear and cytoplasmic changes, indicative of a functionally active state.

4. At this early stage of the intoxication by mercuric chloride there occurs a reduction in blood urea. This decrease in blood urea has been associated with the development of degenerative changes in the liver which first make their appearance in the periphery of the liver lobule.

5. Associated with such changes in the liver and prior to the development of the kidney injury there occurs a reduction in the alkali reserve of the blood.

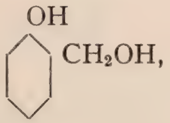
36 (1618)

Mercury saligenin, a new antiseptic.

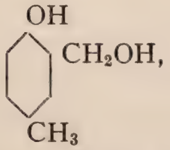
By **Arthur D. Hirschfelder, Merrill C. Hart** and **Frank J. Kucera**.[1]

[*From the Department of Pharmacology, University of Minnesota.*]

The early studies of G. Cohn on saligenin and homosaligenin and the recent investigations of D. I. Macht on benzyl alcohol have demonstrated that these substances are mild antiseptics. We have studied the action of saligenin,



homosaligenin,



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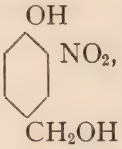
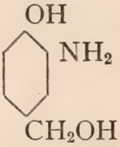
[1] The researches reported in this investigation were made possible by the aid of funds granted by the United States Interdepartmental Social Hygiene Board for the discovery of more efficient medical measures in the treatment and prevention of venereal diseases.

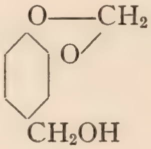
parahydroxymeta nitrophenylcarbinol,



parahydroxymetaaminophenylcarbinol ("Edinol"),

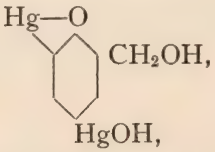


and have found that all of these substances are weak antiseptics. One or two per cent. solutions kill the bacillus coli, staphylococcus albus, streptococcus hemolyticus pneumococcus and gonococcus after 60 minutes exposure. Piperonyl alcohol,



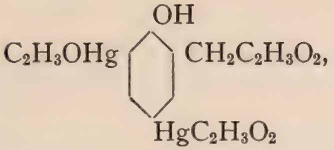
is still weaker.

We have prepared a mercury compound of saligenin,

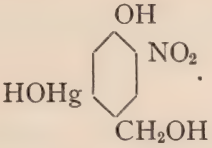


whose sodium salt is water soluble. Solutions of this compound possess about the the same antiseptic power as $HgCl_2$, *i.e.*, 1:10,000 solutions kill staphylococcus, bacillus coli and gonococcus in five minutes and streptococcus hemolyticus in ten minutes in bouillon cultures, while in beef serum bouillon 1:500 solutions kill in five minutes. Mercury saligenin is not a dye, and it is much less irritant to mucous membranes than is $HgCl_2$, so that a 1:1000 soluion can be held in the urethra for five minutes without burning or subsequent irritation. We are therefore testing it clinically in anterior gonorrheal urethritis with encouraging results.

An acetate of this compound,



has also been prepared. Its water soluble sodium salt has the same antiseptic action as that of the mercury saligenin, but no greater. We have also prepared the mercury compound of parahydroxymetanitrophenylcarbinol which yields a monomercury derivative, probably



The sodium salt of this compound has the same antiseptic strength as to the other two mercury compounds.

Attempts at the chemotherapy of trypanosomiasis (brucei) and spirillosis (obermeyeri) in rats by the use of saligenin, piperonyl alcohol and mercury saligenin have thus far yielded only negative results.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

One Hundred Eleventh Meeting.

Rockefeller Institute for Medical Research, New York City,
December 15, 1920.

President Calkins in the chair.

37 (1619)

A pharmacodynamic analysis of cocain action of the cerebrum.

By **D. I. MACHT** and **WM. BLOOM.**

[*From the Pharmacological Laboratory, Johns Hopkins University.*]

The effect of cocain and its chemical components was studied on the intelligent behavior of albino rats in the circular maze. 25 rats were used altogether in the investigation. The animals were trained in the circular maze until they were able to find their way from the entrance to the center without making any errors and in the shortest period of time. The drugs studied were then injected subcutaneously and the effect of the same studied after absorption. It was found that one milligram of cocain produced a marked depression on the behavior of the rats as indicated by incoördination, slowness of movement and loss of memory and intelligence. Smaller doses (one thirtieth to one tenth milligram) also produced distinct depression as indicated by the time of performance and the number of errors made.

An effort was made to ascertain whether cocain produced a primary stimulation of the cerebrum. For this purpose very small doses of cocain were injected. It was found that minute quantities of the drug either failed to produce any effect or produced depression and in no case was there a primary stimulation noted.

Injection of ecgonin-hydrochloride and benzoyl-ecgonin produced no effect on the behavior of the rats even when administered in doses much larger than that of cocain. Injections of sodium benzoate solution produced no effects. Neither was there any depressant or stimulating effect noted after injections of small doses of methyl alcohol solution (1 per cent.).

Various mixtures of ecgonin hydrochloride, benzoyl-ecgonin, sodium benzoate and methyl alcohol in different proportions were found to produce very little effect on the behavior of rats. As a result of the various experiments it is therefore concluded firstly, that cocain, as such, exerts a depressant action on the intelligent behavior of albino rats, secondly, that in no cases even after minute doses of the alkaloid was there a primary stimulation noted and thirdly, that the various components into which the cocain molecule can be split up, when injected individually or as a simple mixture of each other, do not cause the same action as their chemical combination in the form of cocain. The complete data of this investigation will appear in the *Archives Internationales de Pharmacodynamie et de Therapie.*

38 (1620)

Improved methods for staining spirochaeta pallida in tissue.[1]

By **ALDRED S. WARTHIN** and **ALLEN C. STARRY.**

[*Ann Arbor, Michigan.*]

The two methods outlined here present the first satisfactory ones devised for the staining of spirochetes in single sections of tissue mounted upon cover-glasses. We consider them of great value for the following reasons: They are more certain than the Levaditi method of staining in bulk; the time required is shortened to hours, instead of the days required by that method; they have a much greater applicability to practical diagnostic work in that they can be used for single sections, thus permitting a closer control of histological findings.

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[1] Researches conducted under a grant from the Interdepartmental Social Hygiene Board, Washington.

1. WARTHIN AND STARRY'S COVER-GLASS METHOD.

1. Fix tissues in 4 per cent. formol.
2. Wash thoroughly in distilled water.
3. Imbed in paraffin (alcohol, xylol, paraffin).
4. Cut; mount sections on cover-glasses with albumin fixative.
5. Remove paraffin from section (xylol, alcohol, water).
6. Place cover-glass in a saturated solution of ferric alum, or a 4 per cent. solution of ferrous ammonium sulphate, in incubator for 1 to 2 hours.
7. Wash in distilled water.
8. Rinse cover-glass with section in a 2 per cent. silver nitrate solution. Cover section with another perfectly clean cover-glass which has also been rinsed in the silver solution, so that the cover-glasses are held together by capillary attraction. Then place them carefully on the bottom of a wide-mouthed dark bottle covered with black paper, and cover them with the silver nitrate solution. Cork tightly, and put into incubator for 3 to 24 hours.
9. After impregnation pour off the silver nitrate solution and rinse in distilled water without removing cover-glasses from bottle, by pouring the water into the bottle, shaking gently, and then pouring off.
10. Pour reducing fluid (pyrogallic acid, 4 grams; 40 per cent. formol, 5 cc.; distilled water, 100 cc.) into the bottle. See that fluid passes between cover-glasses by pressing upon them with a glass rod, or by shaking. Reduction is almost instantaneous; it should occur evenly over the section or brown lines will result. After 2 to 3 minutes remove cover-glasses, separate, wipe off with a cloth any precipitate on the albumin fixative about the section, taking care not to touch the latter.
11. Wash in distilled water.
12. Dehydrate in absolute alcohol, clear in xylol, then balsam.

The reduced section should have a faint, dull brownish-yellow color. If the color is bright yellow the organisms will be poorly stained. The spirochetes should have a deep reddish brown color contrasting sufficiently well with the background, if the procedure has been successful.

2. WARTHIN AND STARRY'S SILVER-AGAR COVER-GLASS METHOD.

1. Fix tissue in 4 per cent. neutral formol.

2. Imbed in paraffin (absolute alcohol, xylol, paraffin).

3. Cut, mount sections on cover-glasses with albumin fixative.

4. Remove paraffin (xylol, alcohol, water).

5. Rinse cover-glass with section in 2 per cent. silver nitrate; cover wet section with another perfectly clean cover-glass, so that they are held together by capillary attraction; then place them carefully in a bottle of 2 per cent. silver nitrate, and place in incubator for 30 to 60 minutes; then remove the silver nitrate and separate cover-glasses. They must not separate while in the solution.

6. Place cover-glass with section in the following reducing mixture:

2 per cent. silver nitrate solution	3 cc.
Warm glycerin	5 cc.
Warm 10 per cent. aqueous gelatin	5 cc.
Warm 1½ per cent. agar suspension	5 cc.
5 per cent. aqueous hydroquinone solution	2 cc.

7. Reduce until section is a light reddish brown (several seconds); remove and rinse in 5 per cent. sodium hyposulphite solution.

8. Rinse in distilled water. Clean off with a cloth any precipitate on the cover-glass.

9. Absolute alcohol-xylol-balsam.

The mounted section should have a light reddish brown color; if too deep brown or black the spirochetes will not be sufficiently contrasted. They should appear dark reddish brown to jet black against a very light brown background.

In some cases of poor fixation of the tissue with poor staining of the spirochetes we have found that if the sections before being put into the silver solution are treated for several minutes in a 1 per cent. solution of uranium or copper nitrate, or a 0.5 per cent. solution of ferric alum, the tissue-reaction is changed in some way so that good staining results. These solutions do not act uniformly in all cases. After their use the section must be thoroughly washed in distilled water before it is put into the silver nitrate solution.

39 (1621)

The effects of yeast vitamine water-soluble B on plant cell-masses and on biocolloids.

By **D. T. MACDOUGAL.**

[*From the Desert Laboratory, Tucson, Arizona.*]

One of the most clearly outstanding results to be derived from my measurements of imbibition by cell-masses, and of swelling of biocolloids (agar-pentosan mixtures) is that solutions which promote growth in plants increase hydration in living and dead cell-masses and of agar or agar protein mixtures.

As the first step in testing the effects of vitamine, water-soluble B on the growth of some higher plants, its action in hydration was measured on a variety of materials at 15° C. at the Coastal Laboratory, Carmel, California, July to October, 1918. A solution of 1 part in a thousand in distilled water from a preparation furnished by Dr. Isaac Harris was used. Even when prepared under chemically clean conditions and kept in the dark at 15° C. deterioration began within 40 hours and hence freshly made solutions were used, which tested by acidity by the indicator method showed a P_H of 5.25.

The auxograph was used in taking the measurements, trios of sections or of roots being placed in stender dishes into which the solutions were poured. Taking the swellings in water as 100 hydration increases were measured as follows:

Potato tubers, young, sections	75
Potato tubers, large, sections	230
Squash fruits, young, sections of pulp	110
Squash fruits, mature, sections of pulp	115
Orange seedlings, root tips, living	150
Orange seedlings, root tips, dried	120
Corn root tips, small, living	88
Corn root tips, large, living	78
Corn root tips, large, dried	180
Strawberry root tips, living	133
Sunflower stems, sections of pith, mature, living	150
Opuntia, sections of young joints	94
Opuntia, sections of mature joints	150
Opuntia, dried slices	90

It is to be seen from the above that of living cell masses from eight different plants, five showed a greater hydration capacity in the vitamine solution than in water, two were notably less and one had a swelling capacity only slightly less than in water. Of the five which gave excessive enlargement in a living state, two were tested in mature condition and also found to show a high capacity in the vitamine.

Two of the five which showed excessive swelling in a living condition, repeated this action in a dried condition. Lastly it was found that of the three kinds of cell-masses which did not give the normal hydration in vitamine, two gave an excessive swelling in a dried condition.

Coincidently various colloidal preparations were hydrated in the vitamine solution and the swellings at 15° C. relative to that in water are given below.

Agar	140
Agar and "salts"	73
Agar and soap	132
Agar and potassium oleate	80
Agar 3, gelatine 2	136
Agar and phenyl alanine	95
Agar 3, gelatine 2 and "salts"	130
Agar 3, gelatine 2, soap	92
Agar 3, gelatine 3	135
Agar 2, gelatine 3	143
Agar 2, gelatine 3 and "salts"	130
Gelatine	163
Gelatine and "salts"	80
Gelatine and soap	92

The "salts" of the above mixtures consisted of 20 cc. KCl at 0.001M; 12 cc. NaCl at 0.001M; 2 cc. CaCl at 0.0001M, added for each gram of air dried material in making up the colloidal mixtures in water before casting and drying. The soap was a well-known brand sold in flakes and was used as one to a thousand in the colloidal preparation to which it was added. The agar used alone was a purified preparation by Dr. Harris, but bacto-agar was used in the preparation containing soap.

Finally a preparation of agar in which one thousandth part of the amino-acid phenylalanine had been incorporated was hydrated in the vitamine solution with results not essentially different from swelling in water, and the incorporation of yeast vitamine in agar apparently did not increase its swelling capacity although such preparations showed a high hydration capacity in balanced solutions of sodium and calcium chloride in which the first substance was used at 0.0001M.

If we now review the above measurements, the following general statements are seen to be supported:

1. A solution of water soluble B, yeast vitamine at 0.1 per cent., $P_H = 5.25$, causes a hydration in excess of that which may take place in water in agar, agar and soap, agar-gelatine, agar-gelatine and salts in various proportions, and in gelatine.

2. Lessened hydration ensues in agar and salts, agar-gelatine and soap, gelatine and salts, and gelatine and soaps.

3. Mature fruits, pith and joints of plants show excessive swelling in vitamine solutions.

4. Dried cell masses of roots and joints of *Opuntia* show excessive swelling in vitamine solution.

5. Lessened hydration ensues in young tubers of potato and roots of corn, a result parallel to those obtained from mixture of agar, gelatine and soap.

6. Increased hydration takes place in root-tips of orange seedlings and of strawberry plants.

The consideration of these results shows that the effects of this vitamine may not be attributed simply to included amino acids of which nucleic is the most prominent in this instance, nor may they be ascribed to the action of the hydrogen ions at the given concentration, nor to the extremely minute proportions of salts which might be present.

It is suggested that their accelerating action on such unlike colloids might be due to action of various components inert to each other and with effect on but one of the main components of the biocolloids of the mixtures or of living matter.

40 (1622)

Differential survival of male and female dove embryos in increased and decreased pressures of oxygen: A test of the metabolic theory of sex.

By OSCAR RIDDLE.

[*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

Several kinds of evidence have been accumulated which indicate a metabolic difference between the ova (egg-yolks) which give rise to the two sexes in doves. A corresponding difference was also found in more limited investigations of adults of the two sexes. Our previous work has rather consistently indicated that female-producing eggs and female adults have a lower metabolism, males a higher metabolism. Since no metabolic studies upon male and female embryos have hitherto been made this study was carried out as a further necessary test of the complete applicability of the metabolic theory of sex to pigeons.

A method or means of measuring the metabolic differences between embryos of the two sexes is an enormously difficult thing to devise; and probably no plan is devisable at present which does not involve very much work. The many sources of error and difficulty in any attempt to measure heat-production, O_2-consumption of CO_2-production became embarrassingly evident when the unmeasurable and continually changing mass of the embryonic tissue is considered. The plan adopted by us was to subject—during one complete year—all or practically all of the embryos produced by the ring-doves and common pigeons of our collection to reduced and increased concentrations of oxygen (or to expose them to protracted periods of cold) and make such measurements and records as would probably reveal any relation of sex to survival under these conditions. Theoretically, if female embryos have a lower metabolism, *i.e.*, lower minimum oxygen requirement than males, the female embryos should withstand diminished pressures of oxygen somewhat better than male embryos. Similarly—since we had earlier learned that high pressures of oxygen result in the death of some embryos—the male embryos should be somewhat better able than female embryos to withstand an increased concentration of oxygen. Again, if males have a higher metabolism than females the reduced metabolism induced by cooling should perhaps prove more harmful to the male embryos.

Embryos aged 3 minutes to 12 days have been used; but most frequently the age was between 1 hour and 4 days. Increased concentrations of oxygen varying from 26.8 per cent. to 96.6 per cent., and decreased concentrations varying from 18.3 per cent. to 0.15 per cent., were used. The time during which embryos were subjected to the altered pressures of oxygen varied from one to five days. For 0.15 per cent. O_2 the time was 15 minutes to 8 hours. Embryos were normally incubated under birds except during the period of treatment when moisture and temperature (39.4°) conditions were carefully controlled in the experimental chamber. In survival tests under "cooling" the eggs were either left at cold room temperature (50°–65° Fahr.) from 10–13 hours daily during several days or were placed at ice-box temperatures (8°–15° C.) during 18–24 hours. Sixty-five groups were tested in oxygen, 12 in cooling.

The age of the embryo has been found the most important factor in survival under alteration of the gaseous environment. Older embryos are most affected by reduced pressures of oxygen; younger embryos by increased pressures of oxygen. It is probable that the adequacy or thickness of the shell is also a factor in such survival. This adequacy of the shell has been painstakingly measured in all of the treated embryos.

The sex data from the reduced oxygen series have one notable defect. Eight embryos in which color was sex-linked were killed at an age when they should have shown eye-pigment or the lack of it which would declare their sex. Unfortunately, greatly reduced pressures of oxygen have been shown[1] wholly to prevent the post-mortem formation of this pigment and we have evidence that it at least interferes with its formation in these embryos. These eight embryos therefore could not be definitely classified and are omitted from the table. Embryos of known sex were not killed in about one half of the experiments. The lower half

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[1] Riddle and La Mer, *Amer. Jour. Physiol.*, 1918, xlvii, p. 103.

SUMMARY OF SEX DATA FROM OXYGEN AND COOLING EXPERIMENTS.

Complete Data

Nature of Treatment.		No. of Embryos.	Embryos.				Sexes.								Surv'd. Sex Unknown.
			Killed.	Died very Soon.	Survived.	No Test.	Killed.		Survived.		Hatched.		Total.		
							♂	♀	♂	♀	♂	♀	♂	♀	
Incr. O_2.	Totals.	868	136	213	516	3	16	13	271	209	254	198	287	222	36
	Per cent. . . .	—	15.7	24.5	59.3	0.3	5.6	5.9	94.4	94.1	88.5	88.8	129.6	100	7.0
Decr. O_2.	Totals.	792	284	64	434	10	34	22	190	204	176	190	224	226	40
	Per cent. . . .	—	35.9	8.1	54.8	1.3	15.2	9.7	84.8	90.3	78.6	84.1	99.1	100	9.2
Cooling.	Totals.	440	212	—	226	2	34	21	106	113	—	—	140	134	7
	Per cent. . . .	—	48.2	—	51.4	0.4	24.3	15.7	75.7	84.3	—	—	104.5	100	3.1

Only Experiments Showing "Sexed" Individuals Killed.

Nature of Treatment.		No. of Embryos.	Embryos.				Sexes.								Surv'd. Sex Unknown.
			Killed.	Died very Soon.	Survived.	No Test.	Killed.		Survived.		Hatched.		Total.		
							♂	♀	♂	♀	♂	♀	♂	♀	
Incr. O_2.	Totals.	450	86	152	210	2	16	13	107	79	98	74	123	92	20
	Per cent. . . .	—	19.1	33.8	46.7	0.4	13.0	14.1	87.0	85.9	79.7	80.4	133.7	100	9.5
Decr. O_2.	Totals.	448	193	32	215	8	34	22	83	109	74	101	117	131	3
	Per cent. . . .	—	43.1	7.1	48.0	1.8	29.1	16.8	70.9	83.2	63.2	77.1	89.3	100	10.7
Cooling.	Totals.	334	187	—	146	1	34	20	69	73	—	—	103	93	4
	Per cent. . . .	—	56.0	—	43.7	0.3	33.0	21.5	67.0	79.5	—	—	110.8	100	2.7

of the table has therefore been constructed to eliminate those unrepresented experiments.

The table supplies a summary of the chief data obtained concerning sex. It will be understood that the sex of many embryos which were killed, and even of some which survived treatment but died too soon thereafter, could not be ascertained. It is clear that embryos which survive treatment with increased oxygen are about equally capable of completing the remainder of their embryonic development (hatching) regardless of sex. Perhaps the ability to "hatch," after proved survival, should not be considered as within the range of effects of treatment; but these data are given. The quite different sex ratios obtained from the increased oxygen experiments (129.6 ♂♂: 100♀♀) and from the decreased (99.1♂♂ : 100♀♀) is perhaps good evidence that the large number of killed and "unsexed" embryos of the first group contained disproportionate numbers of females, while the killed embryos of the second group probably included more males. The sex ratio from embryos subjected to cooling is of such quantity as to leave it a matter of doubt as to whether the killed and "unsexed" embryos differed in sex ratio from the survivors. This ratio nevertheless markedly differs from that obtained from increased oxygen. The known sex of the killed and of the survivors of this group also indicate clearly that the advantage here rests with the females.

The results are not wholly decisive but give some evidence that sex is also a factor in survival; further, that it is the males which best survive increased pressures of oxygen and females which best survive decreased pressures and cooling. Nearly all of the possible comparisons give the above result. The few figures which are opposed or seem questionable are placed in italics in the table.

41 (1623)

Preparation of collodion sacs for use in bacteriology.

By FREDERICK L. GATES.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City*]

A standardized method was described by which collodion sacs suitable for intraperitoneal incubation and for other bacteriological experiments may be produced in large numbers, sterilized, and handled with convenience and the minimum danger of contamination.

Following the procedure of Prudden and McCrae, 1900, as modified by Harris, 1902, the collodion sacs are made on a gelatin capsule foundation which is then dissolved out with hot water. The essentials of the method are the alcohol treatment of the collodion membranes, as recommended by Brown, 1915, and the protection of the sacs in individual glass containers before and after use. Various factors influencing permeability have been subjected to experiment.

Quantitative experiments on the dialysis of sodium chloride indicate a uniformly high degree of permeability but the permeability conferred by the alcohol treatment is lost during heat sterilization if the membrane was previously allowed to dry. Simple tests with other substances show that the sacs are permeable to gases in solution, to inorganic salts, to dextrose, to certain protein-split products which are nutritive to bacteria, and to certain toxic products of bacterial metabolism, but they hold back antibodies, unsplit proteins, and formed elements such as bacteria and body cells.

The preparation of the sacs is described in detail in the forthcoming (January, 1921) number of the *Journal of Experimental Medicine.*

42 (1624)

Factors influencing anaërobiosis with special reference to the use of fresh tissue.

By **FREDERICK L. GATES** and **PETER K. OLITSKY.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

With methylene blue as an indicator, we have studied the influence of certain elements in promoting or in hindering the development of anaerobic conditions in tissue cultures.

As a result of our experiments, we have come to the following conclusions:

Liquid paraffin oil, used extensively as a seal for anaërobic cultures and in gas analysis, has very little value in inhibiting the access of oxygen. Solid vaseline, on the other hand, forms an effective oxygen-resisting seal. The difference is due to the physical states of the substances at incubator temperature.

Fresh kidney tissue is an active reducing agent and quickly decolorizes methylene blue in its vicinity. The reducing effect of fresh kidney tissue is relative to the amount used. As a reducing agent, at least 0.6 gm. per tube is required for the establishment of an adequate oxygen free zone.

Culture media may be classified as reducing or non-reducing. Those containing dextrose or peptone in a faintly alkaline solution belong to the former class. Ascitic fluid and dilute serum belong to the latter class, for their content of reducing substances is practically insignificant. For the prompt establishment of strictly anaërobic conditions these media require the addition of reducing substances such as dextrose, peptone, or kidney tissue aided by an effective seal or an anaërobic jar.

Semisolid media effectively inhibit the penetration of oxygen to the depths of the tube, but they likewise limit the diffusion of reducing substances and presumably of nutrient substances from imbedded kidney tissue.

The length of the column of medium is of minor importance under a vaseline seal.

43 (1625)

Generalized infection in syphilitic rabbits resulting from the inadequate salvarsan therapy.

By **J. BRONFENBRENNER** and **M. J. SCHLESINGER.**

[*From the Department of Preventive Medicine and Hygiene, Harvard University Medical School, Boston.*]

In the course of study of the spirocheticidal action of salvarsan in vitro it was observed that in very low concentrations, instead of exerting inhibiting action, salvarsan markedly stimulated the growth of spirochetes.[1] Later, on the bases of this observation one of us recommended the use of minute amounts of salvarsan in the medium for isolation of spirochetes in vitro.[2] At the same time it was suspected that introducing minute amounts of salvarsan might also stimulate the growth of spirochetes in vivo. Accordingly a number of male rabbits with experimental syphilitic orchitis as well as two infected females were treated with varying amounts of salvarsan (from 0.004 gm. per kilo down to 0.000004 gm. per kilo) and several of them developed generalized infection, whereas controls treated with large amounts (0.03 gm. per kilo to 0.005 gm. per kilo) or those left untreated showed no tendency to generalized lues during at least 14 months following the date of the experiment. In addition to the involvement of mucous membranes, skin and bones two rabbits developed keratitis. In one rabbit at the autopsy was found a gumma of the liver which was confirmed as such by several pathologists. One of the infected females lost her young twice and the progeny of the third pregnancy was distinctly inferior and all of the four young died within a month after birth.

The work reported above was carried out at the Laboratories of the Western Pennsylvania Hospital, Pittsburgh, in 1914–1916. While a brief verbal mention of these findings was made at the time[3] we have not thus far communicated our observations in print desiring first to confirm them on a larger number of animals. In view of the growing tendency to decrease the dosage of salvarsan in the treatment of syphilis, we feel it useful to call attention to the facts observed by us.

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[1] Bronfenbrenner and Noguchi, *Jour. Pharm. and Exp. Therapeutics*, 1913, l, p. 333.

[2] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1915, xii, p. 136.

[3] Address before the Medical Society of St. Louis, January, 1915.

44 (1626)

The specific electrical conductivity of the tissue fluids of desert Loranthaceae.

By **J. ARTHUR HARRIS** and **A. T. VALENTINE.**

[*From the Station for Experimental Evolution, Cold Spring Harbor, L. I.*]

MacDougal and Cannon[1] and MacDougal[2] suggested some years ago that the osmotic concentration of the tissue fluids of the two organisms is one of the fundamental variables in the relationship between plant parasite and host. Senn[3] has published one plasmolytic determination indicating higher concentration in a *Viscum* than in the leaves of the host tree and has secured similar results with other phanerogamic parasites. In the Jamaican montane rain-forest the concentration of the tissue fluids of the parasitic Loranthaceae is in general higher than those of the host.[4] The same relationship has been found to obtain in desert Loranthaceae.[5]

As far as we are aware the relative electrolyte contents of the tissue fluids of parasite and host have not been determined heretofore.

In August, 1920, we had the opportunity while carrying out work for the U. S. Department of Agriculture at Sacaton, Arizona to measure the specific electrical conductivity, K, as well as the osmotic concentration in atmospheres, P, calculated from the freezing point lowering, Δ, of the expressed sap of the leaves of the host trees and of the stems of the leafless *Phoradendron cali-*

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[1] MacDougal and Cannon, Pub. Carn. Inst. Wash., 1910, No. cxxix, P. 3, 25–49.

[2] MacDougal, *Bot. Gaz.*, 1911, lii, 249–260; *Bull. Torr. Bot. Club*, 1911, xxviii, 54–55, 473–480.

[3] Senn, *Verhandl. Naturf. Ges. in Basel*, 1913, xc, 179–183.

[4] Harris and Lawrence, *Amer. Jour. Bot.*, 1916, iii, 438–455.

[5] Harris, *Mem. Torr. Bot. Club*, 1918, xvii, 307–315.

fornicum parasitic on the leguminous trees *Acacia greggii* and *Olneya tesota* and of the leaves of the leafy *P. cockerellii* parasitic on *Populus wislizeni*, *Salix wrightii* and *Fraxinus attenuata*.

Sap was extracted after antecedent freezing of the tissues in an ice and salt mixture[1] to facilitate extraction[2] and the constants determined on the centrifuged sap.

Table I shows the average values of Δ and of P as determined from a published table.[3]

TABLE I.

Freezing Point Lowering Δ, and Osmotic Concentration, P.

Parasite and Host.	Δ			P		
	Parasite.	Host.	Difference.	Parasite.	Host.	Difference.
P. Californicum						
on *Acacia greggii*	2.81	2.21	+0.60	33.66	26.57	+7.09
on *Olneya tesota*	2.25	2.10	+0.15	26.98	25.24	+1.74
P. Cockerellii						
on *Populus wislizeni*	1.92	1.84	+0.08	23.05	22.04	+1.01
on *Salix wrightii*	2.08	1.74	+0.34	24.98	20.88	+4.10
on *Fraxinus attenuata*	2.20	1.96	+0.24	26.47	23.47	+3.00

For each comparison the osmotic concentration of the tissue fluids of the parasite is higher than that of the host. Thus the results of earlier investigations in Jamaica and Arizona are confirmed.

TABLE II.

Specific Electrical Conductivity, $K \times 10^5$, and the Ratio of K to Δ, $K/\Delta \times 10^5$.

Parasite and Host.	K			K/Δ		
	Parasite.	Host.	Difference.	Parasite.	Host.	Difference.
P. Californicum						
on *Acacia greggii*	2242	1509	+ 733	831	682	+149
on *Olneya tesota*	2471	2192	+ 279	1103	1052	+ 51
P. Cockerellii						
on *Populus wislizeni*	3061	1990	+1071	1598	1094	+504
on *Salix wrightii*	3101	1582	+1519	1488	908	+580
on *Fraxinus attenuata*	2399	1461	+ 938	1091	749	+342

The constants for specific electrical conductivity and for the ratio of electrical conductivity to freezing point lowering appear in Table II. This shows that electrical conductivity, like freezing-point lowering, and the ratio K/Δ is higher in parasite than in host.

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[1] Gortner and Harris, *Pl. World*, 1914, xvii, 49–53.

[2] Dixon and Atkins, *Sci. Proc. Roy. Dublin Soc.*, 1913, N. S., xiii, 422–423; Gortner, Lawrence and Harris, *Biochem. Bull.*, 1916, v, 139–142.

[3] Harris and Gortner, *Amer. Jour. Bot.*, 1914, i, 75–78.

Thus it appears that there is some mechanism not as yet determined by which the mistletoe accumulates and retains in solution larger quantities of dissociated salts or organic acids than does the host.

It is possible that higher transpiration from the parasite might result in the accumulation in a purely mechanical manner of larger amounts of salts from the transpiration stream, but this is merely a suggestion requiring further investigation.

45 (1627)

Chemical and molecular behavior of casein.

By **JACQUES LOEB.**

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

Two kinds of casein were used, both prepared according to Van Slyke and Baker, the one from skimmed milk, the other from a solution of purchasable "pure casein." Since albumin is soluble near a P_H of 4.7 while casein is not, in both cases, casein practically free from albumin was obtained.

It was possible to show that when HCl or H_3PO_4 are added to isoelectric casein, three times as many c.c. of 0.1N H_3PO_4 as of 0.1N HCl are required to bring 1 gm. of isoelectric casein in a 1 per cent. solution to a given P_H. On the other hand, it required equal numbers of c.c. of 0.1N $Ca(OH)_2$ or $Ba(OH)_2$ as of 0.1N KOH or NaOH to raise 1 gm. of isoelectric casein in 100 c.c. solution to the same P_H. Hence H_3PO_4 combines with casein in molecular proportion while $Ca(OH)_2$ and $Ba(OH)_2$ combine in equivalent proportions with casein. In other words, acids and alkalies combine with casein by the same purely chemical forces of primary valency as they combine with crystalloids. The same fact had been shown by the writer for the combination of these acids and alkalies with gelatin and crystalline egg albumin.[1]

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[1] J. Loeb, *Journ. Gen. Physiol.*, 1918–19, i, 483, 559; 1920–21, iii, 85; *Science*, 1920, lii, 449.

According to the writer's experiments on these latter two proteins it was expected that the curves for the osmotic pressure and viscosity of 1 per cent. casein chloride and casein phosphate solutions should be practically identical when plotted over P_H as abscissae. The experiments confirmed this expectation.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

One hundred twelfth meeting.

College of Physicians and Surgeons, New York City, January 19, 1921. President Calkins in the chair.

46 (1628)

Comparative study of ethanol, caffeine and nicotine on behavior of albino rats.

By **D. I. MACHT** and **WM. BLOOM.**

[*Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

The effects of ethanol, caffeine and nicotine tartrate were studied on albino rats in the circular maze. The rats were first trained to solve the maze problem perfectly, that is to find their way to the center of the maze by the shortest route, without making any errors and in the shortest period of time. They were then injected subcutaneously or intraperitoneally respectively with solutions of the above drugs, and the effects of the drugs on behavior were noted. Controls were made by injecting rats with solutions of sodium chloride and with distilled water. No improvement in the running time of the animals was noted after small doses of the drugs. All of the drugs when given in sufficient quantity produced depression of the animals, as manifested by slower movements, neuromuscular incoördination, loss of memory-habit, and number of errors committed. It was found that the smallest dose of caffeine required to produce depression, that is to impair the efficiency of the rats' behavior in the maze, was 10 mgs. for a rat weighing on an average 150 grams. The smallest dose of nicotine tartrate to produce depression was 0.02 mg. (equivalent to 0.007 mgs. of nicotine). The smallest dose of ethanol to produce depression, when injected in the form of 4 per cent. solution was about 80 mgs. by weight.

47 (1629)

The effect of prostatectomy on the behavior and learning of albino rats.

By **D. I. MACHT** and **WM. BLOOM.**

[*Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

The prostate gland of the rat is proportionately to the size of the animal much larger than that of man. It can also be completely extirpated with comparative ease. These facts render rats especially suitable as subjects for the inquiry into the question of the internal secretion of that gland. In the present investigation an attempt was made to throw light on the relationship between prostatectomy and mental efficiency, the existence of which seems to be supported by some clinical evidence.

Two series of experiments were conducted on white rats in the circular maze. In the first series of experiments a number of rats were trained to solve the maze problem by finding the way to the center of the maze, by the shortest route, without any errors, and in the shortest period of time. They were then prostatectomized under ether anesthesia, allowed to recover, and their behavior was studied subsequently. Control experiments were made on other rats of the same series and same ages, which were also anesthetized and on which laparotomy was performed but without removal of the prostate.

In the second series of experiments, young adult male rats were prostatectomized without previous training in the maze, and control laparotomies were also made as before. The animals were allowed to recover and were kept in their cages for periods ranging from 5 to 9 weeks. Then they were trained in the maze and a comparison was made between the learning time of the prostatectomized and control rats.

An analysis of all the data obtained in the two series of experiments revealed that the extirpation of the prostate exerted no influence either on the behavior or the rate of learning of the animals. Fuller data to appear in the *Journal of Urology.*

48 (1630)

A substance toxic to guinea pigs in the blood of infants with "intestinal intoxication."

By OSCAR M. SCHLOSS.

[*From the Department of Pediatrics, Cornell University, New York City.*]

Cases of so-called "intestinal intoxication" can be divided into two groups. In the first group there is a history of preceding nutritional disturbance with diarrhea of days or weeks duration. The onset of toxic symptoms is often gradual. The infants are greatly wasted and the tissues show signs of water loss. The blood concentration may be slightly increased but is often normal. The mental state is best described as somnolent. Pronounced nervous symptoms are absent.

In the second group the onset is usually sudden and preceding nutritional disturbances are slight or may not occur at all. Wasting is slight. Diarrhea is usually not severe and in many cases does not occur. One of the striking features of this group is the presence of pronounced nervous symptoms. Convulsions are common and the patient is in deep coma with marked involuntary movements and muscular twitchings. The blood of these infants is much concentrated. The clinical picture, the complete anuria and the high non-protein nitrogen of the blood in these cases strongly suggest a relationship to uremia. The work of Foster on uremia suggested the possibility that the blood of infants with this severe type of intoxication might show the presence of a toxic substance.

8 to 15 c.c. of blood serum or citrated plasma were dialyzed through collodion against from 50 to 100 c.c. of water for 12 to 24 hours. The dialysate was rapidly concentrated in a current of air at a temperature below 40° to a volume of 3 to 5 c.c. and injected into the peritoneal cavity of guinea pigs.

There were 13 experiments. In 6 animals there were no symptoms of consequence. Seven of the animals showed distinct symptoms. Two died within two hours. Three of the remainder showed pronounced symptoms. Two of these died in four to five hours respectively, the third recovered in twelve hours. One animal showed very slight symptoms but died in twelve hours. One showed moderate symptoms, but recovered completely in two hours.

The symptoms presented by the animals were great uneasiness, scratching of the nose, bucking movements, paralysis and pronounced dyspnea. There was usually a fall of temperature of 5 to 7 degrees F. Autopsy of the 4 animals which died within 5 hours showed great distension of the lungs and peritoneal and pericardial hemorrhages. No pathological changes were apparent in the animal which died in 12 hours.

Two series of control were done. (1) The blood of 17 practically normal infants was treated as outlined and injected into guinea pigs. None of the animals developed symptoms. (2) The blood of 13 infants suffering from acute infections was tested. Six had pneumonia, 3 tonsillitis and 4 infectious colds with otitis media. The results were negative. Three of the infants with pneumonia had diarrhea.

These experiments seem to indicate that the blood of infants with a type of intestinal intoxication may show the presence of a substance toxic to guinea pigs. No evidence is available at present to indicate the nature of this substance or its relation to the disease.

49 (1631)

General effects of increased and decreased pressures of oxygen on dove embryos.

By **OSCAR RIDDLE.**

[*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

Compared with hatched young or adults the dove embryo has very inferior powers of adjustment to either high or low oxygen pressures. The earliest stages, in fact, wholly lack the usual or other apparent mechanisms of respiratory compensation. Contrary to permissible inferences from the few studies hitherto made with older embryos only, it is found that the embryos in the youngest stage are usually much affected by prolonged high concentrations of oxygen. Some of these embryos are killed by subjection to high oxygen pressures during 24 hours; others may temporarily survive—as shown by heart-beat—for more than four days, but these embryos usually fail to develop blood pigment, become highly abnormal and remain of very small size; some of these abnormal embryos practically complete their development—producing "monsters" in which the head and eyes are most often affected. That such abnormal embryos and monsters arise only from very young embryos, of 2.2 days or less, is indicated by the data of Table I.

TABLE I.

SHOWING THAT THE PRODUCTION OF DEVELOPMENTAL ABNORMALITIES AND "MONSTERS" BY INCREASED OXYGEN PRESSURES (95.8 PER CENT., DURING 23 HOURS) IS CLOSELY LIMITED TO THE YOUNGEST EMBRYOS TREATED.

Age (in Days) of Embryo.	Killed.	Survived.	Normal or Abnormal (for Survivors).	Age (in Days of Embryos.	Killed.	Survived.	Normal or Abnormal (for Survivors).
7.5		+	Normal	4.2	*	*	
7.5	*	*		4.2	*	*	
7.2	*	*		4.0	*	*	
7.0	*	*		3.5	*	*	
7.0		+	Normal	2.5		+	Normal
6.5	*	*		2.5	*	*	
6.2	*	*		2.5	*	*	
6.2	*	*		2.4	1	1	
6.0		+	Normal	2.2	*	*	Abnormal
6.0		+	Normal	2.0	*	*	
6.0		+	Normal	2.0	2	2	Abnormal
5.5	*	*		1.5	+		
5.5	*	*		1.2	2	2	Abnormal
5.5	*	*		1.2	2	2	Abnormal
5.5	*	*		1.2	3	3	Abnormal
5.2	*	*		1.0	2	2	Abnormal
5.2	*	*		1.0	*	*	
5.0		+	Normal	1.0	*	*	
5.0		+	Normal	0.5	?	?	Abnormal
5.0		+	Normal	0.5	?	?	Abnormal
4.5	*	*		0.0		+	Normal
4.2	*	*		0.0	?	?	Abnormal
4.2	*	*		0.0		+	Normal

* Embryo alive at end of experiment but died soon afterward.

[1] Hemoglobin nearly or quite absent, heart beating 50 hours after experiment.

[2] Hemoglobin nearly or quite absent, heart beating 74 hours after experiment.

[3] Hemoglobin nearly or quite absent, heart beating 100 hours after experiment.

It should be stated that monsters rather similar to those induced by the increased oxygen have resulted also from treatment with reduced oxygen pressures and these have all been produced in embryos aged 2 days or less at the time of treatment. However, some of the abnormalities of the types mentioned above are practically absent in series of embryos treated with reduced oxygen. That the monsters are in fact induced by the altered oxygen pressures is adequately shown by our complete data. These data are the result of 80 experiments upon nearly 2,000 embryos of various but precisely known ages, with oxygen concentrations varying from 8.0 to 96.5 per cent., and with duration of treatment extended from 8 hours to 10 days.

In older embryos subjected to increased oxygen the normal amount of hemoglobin is decreased, as indicated by macroscopic examination, and quantities of it appear in solution in the amniotic fluid. Quantitative studies of the changes in the blood pigment of embryos subjected to decreased and increased oxygen pressures may be made later.

During the various stages of its development the embryo utilizes three different respiratory surfaces and these are further associated with from none to several of the usual compensatory mechanisms. Age therefore becomes the chief factor involved in the death or survival of embryos subjected to altered oxygen pressures. This fact is sufficiently shown in Table II. The data of the table also indicate that longer periods of exposure are more lethal than are 24-hour periods; adequate compensatory changes are usually limited to definite stages or ages of the embryo. Tests have shown that the adult bird readily survives 24-hour periods in either 10 per cent. or 95 per cent. oxygen.

Embryos of 8–10 days and older are like hatched individuals in being able to survive the highest percentages of oxygen. The youngest stages best survive decreased pressures of oxygen; for this stage the oxygen may be reduced to 9–10 per cent. (24 hours). It is clear, however, that this "cold-blooded" stage of the bird embryo is unable to reduce the oxygen demands of the tissues to a further reduction of the oxygen supply.

Although the age of the embryo is the most important factor in its capacity to survive an altered pressure of oxygen the data of

TABLE II.

SHOWING THE RELATION OF AGE OF EMBRYO TO SURVIVAL UNDER ALTERED OXYGEN PRESSURES.

Exp. No. 29, 24 Hours, $O_2 = 95.0\%$.			Exp. No. X, 240 Hours, $O_2 = 50.1\%$.			Exp. No. 40, 72 Hours, $O_2 = 12.6\%$.			Exp. No. 39, 24 Hours, $O_2 = 12.4\%$.		
Age of Embryos.	Killed.	Survived.	Age of Embryos.	Killed.	Survived.	Age of Embryos.	Killed.	Survived.	Age of Embryos.	Killed.	Survived.
9.0		+	5.0		+	5.5	+		10.0		+
7.2		+	4.5		+	5.0	+		9.5	+	
6.5	+		3.2		+	4.5	+		9.0	+	
6.2		+	2.5	+		4.2	+		8.2[1]	+	
5.2	+		2.5		+	4.0	+		4.5	+	
5.0		+	2.2		+	3.2	+		4.2	+	
4.5	+		2.0		+	2.5	+		3.5		+
4.2	+		1.2		+	2.5	+		3.2		+
4.0	+		1.2		+	1.2		+	3.2	+	
3.5	+		1.0	*	*	1.2		+	3.0	*	*
3.2	+		0.5	*	*	1.0		+	2.5		+
3.2	*	*	0.5	+		1.0		+	2.5	+	
2.5	*	*	0.5	+		0.0		+	2.2		+
2.2	+		0.3	+		0.0	*	*	2.2		+
1.5	+		0.0	+					2.0		+
1.2	+								2.0		+
1.0	+								1.5		+
0.6	+								1.2		+
0.6	+								1.2		+
0.2	+								1.0		+
0.2	+								0.2		+

* Survived period in altered oxygen pressure but died immediately afterward.

[1] Seven embryos aged 6.5 to 4.5 days, *all killed*, are omitted to save space in table.

the two tables given here clearly indicate that it is not the sole factor. A study of the complete data, one part of which has been previously reported,[1] makes it probable that variations in the permeability of the egg-shell to gases and the sex of the enclosed embryo are such additional factors.

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[1] Riddle, Oscar, *Proceedings of the Society for Experimental Biology and Medicine*, 1920, xviii, 88.

50 (1632)

Maximum values of osmotic concentration in plant tissue fluids.

By J. ARTHUR HARRIS, R. A. GORTNER, W. F. HOFMANN, and A. T. VALENTINE.

[*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y., and the University of Minnesota, St. Paul, Minnesota.*]

The observations of a number of botanists have shown that extremely high concentrations may characterize plant tissue fluids, especially when the plants[1] occur in a highly saline substratum. To Fitting[2] belongs the credit of first demonstrating that extremely high osmotic concentrations are found in some desert plants,[3] although Drabble and Lake[4] and Drabble and Drabble[5] had preceded him in showing the fundamental relationship between environmental conditions and the osmotic concentration of plant tissue fluids.

As early as 1902 Cavara reported cryoscopic determinations on saps of high concentration[6] and in 1905 gave results in full[7] for a large series of determinations made at Cagliari. His maximum values were found in the sap of halophytes growing in localities where the concentration of the soil solution progressed with the advance of the season. He reports freezing point depressions of 7.25° in *Obione portulacoides*, 7.48° in *Salicornia fruticosa*, and 7.25° to 8.50° in *Halocnemum strobilaceum*. His determinations were, however, made on sap extracted without the antecedent treatment necessary to render the tissues permeable as has been shown to be necessary by Dixon and Atkins[8] and others.[9] His constants are, therefore, as pointed out by Atkins,[10] probably submaximum because of incomplete extraction.

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[1] Our present observations apply to the tissue fluids of flowering plants only. No attempt is made here to discuss the concentrations found in such lower organisms as those studied by G. J. Peirce (Pub. Carn. Inst. Wash., 1914, No. 193, p. 47–69) or G. Senn (*Verh. Schw. Naturf. Ges.*, 1911, xciv).

[2] H. Fitting, *Zeitschr. f. Bot.*, 1911, iii, 209–275.

[3] Fitting found a number of species of plants in the North-African deserts, the leaf cells of which were not plasmolyzed by 3 gram molecular KNO_3 solution. Theoretically potassium nitrate of this concentration should be the equivalent of about 100 atmospheres. The technical difficulties of applying the plasmolytic method are such as to lead one to question its value as a means of investigating in a quantitative manner the unusually high concentrations found in desert plants.

[4] E. Drabble and H. Lake, *New Phytologist*, 1905, viii, 189.

[5] E. Drabble and H. Drabble, *Biochem. Jour.*, 1907, ii, 117.

[6] F. Cavara, *Rendic. Congr. Bot. Palermo*, 1902, 66.

[7] F. Cavara, *Contrib. Biol. Veg.*, 1905, iv, 41–84.

Work on the spring flora of the Arizona deserts[11] was probably carried out in a manner to obviate the objections to the preceding studies. In this series the maximum concentrations were found in *Atriplex canescens*, a shrub of the salt spots, in which $\Delta = 5.65$, $P = 67.5$, and in *Mortonia scabrella*, a small shrub of the mesa-like slopes, for which one determination gave $\Delta = 4.78$, $P = 57.2$.

Concentrations of about fifty atmospheres have been demonstrated in the leaf tissue fluids of more or less sclerophyllous trees *Capparis ferruginea* and *Guaiacum officinale* and in those of the succulent-leaved halophytic half shrub *Batis maritima* of the saline coastal flats of Jamaica.[12] Cryoscopic studies on mangrove vegetation[13] have indicated maximum concentrations of about fifty atmospheres in *Avicennia nitida*, although two questionable determinations indicated seventy atmospheres. Using plasmolytic methods, von Faber[14] reports concentrations ranging from 24 to 72 atmospheres in East Indian species of the mangrove association.

During the summer of 1920, while engaged in field operations in collaboration with the Department of Agriculture in the Great Salt Lake region, we had the opportunity of making several hundred determinations of the osmotic concentration of plant tissue fluids by the cryoscopic method. These measurements were made on sap extracted after freezing of the tissues[15] and with such care as to render the results reasonably free from criticism. Such a series, based on species which have for ages been subject to the influence of the highly saline substratum afforded by the bed of the ancient Lake Bonneville, should furnish some indication of the maximum concentration[16] to be found in the leaf tissue fluids of flowering plants.

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[8] H. H. Dixon and W. R. G. Atkins, *Proc. Roy. Dublin Soc.*, 1913, N. S., xiii, 422–433.

[9] R. A. Gortner, J. V. Lawrence, and J. Arthur Harris, *Biochem. Bull.*, 1916, v, 139–142, *pl. 1.*

[10] W. R. G. Atkins, "Some Recent Researches in Plant Physiology," London, 1916, 94.

[11] J. Arthur Harris, J. V. Lawrence, and R. A. Gortner, *Phys. Res.*, 1916, ii, 1–49.

[12] J. Arthur Harris and J. V. Lawrence, *Bot. Gaz.*, 1917, lxiv, 285–305.

[13] J. Arthur Harris and J. V. Lawrence, *Biol. Bull.*, 1917, xxxii, 202–211.

[14] F. C. von Faber, *Ber. Deutch. Bot. Ges.*, 1913, xxxi, 277–281.

[15] R. A. Gortner and J. Arthur Harris, *Pl. World*, 1914, xvii, 49–53.

While high concentrations were demonstrated in a number of species, the highest was found in the typical salt desert half-shrub *Atriplex confertifolia*. It alone will be considered.

Two collections made on the rocky cliffs of Stansbury Island, Great Salt Lake, on July 14 gave freezing point depressions of 6.96° and 7.97°. If we may use the formula of Lewis,[17] upon which published tables of osmotic concentration have been based,[18] these depressions indicate osmotic pressures of 82.9 and 94.7 atmospheres respectively.

The highest concentrations were found in plants growing on the low ridges in the salt-flats[19] along the southern shore of Great Salt Lake. A determination on material collected July 16 gave $\Delta = 6.22$, $P = 74.2$.

On July 18 a determination on plants in about the same type of locality gave $\Delta = 10.00$, $P = 118.5$. Finally, on July 27 a determination made in this locality on the leaves of this species indicated a freezing point lowering of 13.0°. The equation used would indicate a concentration of 153.1 atmospheres.[20]

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[16] A difficulty in work on the leaves of desert plants lies in the fact that the maximum concentrations must be expected during the periods of more extreme drought. During such periods the saps may become concentrated by desiccation merely. We know very little concerning the functional activities of such leaves or whether they are retained after the beginning of a period of more adequate moisture. There is, therefore, the possibility that leaves which are too desiccated to be longer functional may be utilized for determinations and indicate concentrations which are really larger than those in which the metabolic processes of the cells may be normally carried on. We believe that except as specifically indicated, the concentrations here recorded were determined on leaves in fairly normal condition.

[17] G. N. Lewis, *Journ. Amer. Chem. Soc.*, 1908, xxx, 668–683.

[18] J. Arthur Harris and R. A. Gortner, *Amer. Jour. Bot.*, 1914, i, 75–78; Harris, *Amer. Jour. Bot.*, 1915, ii, 418–419.

[19] T. H. Kearney, L. J. Briggs, H. L. Shantz, J. W. McLane, and R. L. Piemisel, *Jour. Agr. Res.*, 1914, i, 365–417, pl. 52–58.

[20] A sample from *Atriplex nuttallii* showed a freezing point lowering of about 14.4°, indicating a concentration of 169.3 atmospheres. The leaves appeared more dried than those of *Atriplex confertifolia*, and we are inclined to await further measurements before accepting this constant.

These determinations show that concentrations measured by a depression of 13.0°, presumably the equivalent of 153 atmospheres, may be found in the tissue fluids of apparently normal leaves.

51 (1633)

The carbohydrate-fat ratio in relation to the production of ketone bodies in diabetes mellitus.

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[*From the Chemical Division, Medical Clinic, Johns Hopkins University and Hospital, Baltimore, Maryland.*]

Since relatively large amounts of fats are used in the construction of maintenance diets in the treatment of diabetes mellitus it is important to know the limits within which fat may be employed with safety. The normal composition of fat demands that carbohydrates shall be simultaneously oxidized. Zeller[1] has shown in two normal men and two normal dogs that of the total calories, the protein intake being kept low, 10 per cent. must be yielded by carbohydrate if 90 per cent. arises from fat in order to prevent the production of the ketone bodies. In commenting on these experiments Lusk[2] calls attention to the fact that it is possible that for the proper oxidation of fat, the end product of which is B-oxybutyric acid, the burning of one triose molecule may be necessary for the normal oxidation of one molecule of B-oxybutyric acid. The attempt has been made in this work to establish the proportion of available carbohydrate to fat when ketone bodies appear in the urine.

Diabetic cases are treated as follows: Freed from sugar and acetone body excretion, sugar tolerance ascertained and then the following experiment. The individual is put on a diet having a protein intake that will enable nitrogen equilibrium to be maintained with the fat and carbohydrate given. During different periods the protein intake is kept constant and the amount of carbohydrate and fat are varied isodynamically, the proportion of carbohydrate being reduced in proportion to the fat until ketone bodies show a definite increase in the urine.

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[1] Zeller, *Archiv. fur Physiologie*, 1914, p. 213.

[2] Lusk, "Science of Nutrition," third ed., pp. 270–271.

The following table shows the percentage relationship of fat and carbohydrate and total available carbohydrate in the diet of the patients at the point in the experiment where the ketone body excretion shows a marked increase.

Case No.	Per Cent. of Total Calories Yielded by			Gms. Avail CH.	Gms. Fat.	Ratio.
	Fat.	CH.	Avail. CH.			
101	79	4	14	60	150.6	1 : 2.5
102	87	4	9	25.1	111.0	1 : 4.4
103	78	11	17	40.9	80.4	1 : 1.9
104	87	3	9	31.4	140.1	1 : 4.5
105	84	5	12	25.4	80.5	1 : 3.2
106	83	5	12	25.6	80.1	1 : 3.2
107 non diabetic	89	4	8	51.6	235.3	1 : 4.6
109	87	3	8	28	129.9	1 : 4.0
110	78.1	5.5	15	36.6	84.1	1 : 2.3[4]

[3] Total available carbohydrate calculated by taking 58 per cent. gms. protein and adding to grams carbohydrate.

[4] Case No. 103 re-admitted seven months later.

The results suggest that the ratio of carbohydrate to fat necessary for complete oxidation of the fat may be about the same as Zeller obtained in normals.

52 (1634)

The nutritive value of extra-yeast bread.

By **PHILIP B. HAWK, CLARENCE A. SMITH,** and **OLAF BERGEIM.**

[*From the Laboratory of Physiological Chemistry of Jefferson Medical College, Philadelphia.*]

Eleven albino rats were placed upon a diet containing besides inorganic salts and butter fat in adequate amounts, a bread made from white flour in the ordinary manner containing the usual amount of yeast, the liquid used in preparing the dough being half milk and half water.

Another group of eleven rats of the same average weights were placed upon a diet similar to the preceding, except that 5 per cent. of dried yeast was added to the flour and some extra fresh yeast added to raise the dough.

The rats on the ordinary bread grew very poorly, gaining on the average only 18 grams in 9 weeks. The rats on extra-yeast bread grew much better, gaining 59 grams on the average in 9 weeks.

The superior nutritive value of the extra-yeast bread was ascribed to its high content of water-soluble B and to the supplemental action of the complete protein of the yeast.

53 (1635)

Evidence for sex-linked lethal factors in man.

By **C. C. LITTLE** and **MARION GIBBONS.**

[*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

Among the points brought to light during the investigations which followed the rediscovery of Mendel's Law of Heredity were two of especial interest to medical men. These were lethal factors and sex-linked inheritance.

A lethal factor is a Mendelian unit which can be carried by a normal individual as can any recessive, but which when present without its normal allelomorph to balance it, causes the death of the individual possessing it. Among those lethal factors demonstrated for mammals is that for yellow coat color in mice. The color of the wild house mouse is called by geneticists "black agouti." It has as an alternative condition or allelomorph, a type in which almost if not all, black and brown pigment has disappeared from the coat leaving only the yellow pigment unmodified. When black agouti mice are crossed together yellows are never produced. When yellows are crossed together however, yellows and black agoutis are produced in a ratio of 2 to 1. If the yellows had been ordinary mendelian heterozygotes, the ratio should have been 3 to 1, but it is clear that the 2 to 1 ratio is the one involved.

The yellows so produced are never homozygous, as they should be in 33 per cent. of the cases, were unmodified mendelian inheritance involved. The obvious hypothesis is that the homozygous yellow individuals start their development, but perish in early embryonic stages. This suggestion was therefore made by Castle and Little (1910) and has since been supported by the histological and embryological findings of Kirkham, Ibsen and others.

The case may be diagrammed as follows:

A^y = yellow
A = black agouti.
(Yellow carrying black agouti) × (yellow carrying black agouti)
$A^yA \times A^yA$.

$$\text{Forms}\begin{cases} A^yA^y \text{ homozygous yellow dies.} \\ 2A^yA \text{ yellows carrying black agouti.} \\ AA \text{ black agouti.} \end{cases}$$

Sex-linked inheritance is slightly more complicated but has been completely demonstrated. The approximate equality of male and female individuals which characterizes most species of the higher animals, is strongly suggestive of the 1 : 1 mendelian ratio. This ratio is obtained in mendelian inheritance when a DR individual is crossed with either a DD or an RR individual. The condition found in mammals suggests that the female is DD, the male DR in constitution. Evidence for this is derived from the peculiar behavior of certain color characters in inheritance. One of the clearest of these cases is seen in cats. Here black and yellow coat color represent alternative or allelomorphic conditions.

When a black female cat is crossed with a yellow male, two classes of progeny are produced. These are black males and tortoise shell (blotched yellow and black) females. There has been a "criss cross" type of inheritance in which all the sons resemble the mother. This case has been explained on the supposition that there is linkage in inheritance between the factors determining yellow or black coat color and the substances designated X, which are supposed to be intimately connected with the determination of sex.

Thus if B^y = the factor for yellow.
B = the factor for black.
BX BX = black female.
B^yX – = yellow male.

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2 B^yX BX = tortoise-shell females.
2 B^yX – = black males.

Especially interesting are the relations of the colors in the two sexes. It will be noted that in the tortoise-shell females *neither* the yellow factor nor the black factor are completely dominant. They both have a share in the color of the individual and may therefore be considered as balancing each other's activity to some extent. In the male however, the black factor is not balanced by any factor for yellow and thus produces a black coat.

In man it has been shown that several characters, among which are hemophilia and color blindness, depend primarily upon mendelizing units carried in the X or sex chromosome. Their inheritance would, if the hemophilic or color blind condition is recessive, be as follows:

H = normal,
h = hemophilia.

HXHX normal ♀ mated with hX – hemophilic ♂:
Offspring; HXhX – normal female (carrying recessive hmophilia).
HX – normal male.

HXhX heterozygous normal ♀ mated with HX – normal ♂.
HXHX = normal female.
HXhX = normal female (carrying hemophilia).
HX – = normal male.
hX – = hemophilic male.

Lethal factors if sex-linked, follow the same type of inheritance. Thus if L = normal, l = lethal.

LXlX = normal female heterozygous, crossed with LX – normal male.

LXLX = normal female.
LXlX = normal female (heterozygous).
LX – = normal male.
lX – = lethal male (dies).

Since any sex-linked lethal factor in man would, by hypothesis, be borne in the same chromosome as the factor for hemophilia or for color blindness or their normal allelomorphs, it would be linked to them in inheritance. If it were closely linked with the normal allelomorph of hemophilia it would cause the death of the great majority of the normal males which in its absence should occur in equal proportions to hemophilic males. (See diagram above.)

In human families with their relatively small numbers, it would not be at all surprising to find that all the sons in such families would be hemophilic and that by the selective elimination of their normal brothers an excess of hemophilic above the 1 : 1 ratio would be produced. The same would hold true for color blindness.

When a tabulation of the data available in Bulloch and Fildes monograph on hemophilia and at the Eugenics Record Office of the Carnegie Institution of Washington was made such an excess was found to exist even after due allowance is made mathematically for the one hemophilic male occurring in each family. The excess of hemophilics over the expected is so great that the odds are greater than one to a thousand million that it is due solely to chance. The actual numbers are 551 observed to 457 expected. The data available on parallel matings of color blindness are much more meager but offer supporting evidence. The odds are greater than 1 to twenty-six that the result is due solely to chance. The observed number of color blind is 106 and the expected number 90.

In both cases, therefore, in spite of the fact that both hemophilic and color-blind individuals are certainly no better fitted for survival than their normal sibs under equal opportunities, *there is an excess of the abnormal types. This clearly suggests the intervention of a sex-linked lethal factor which eliminates the otherwise "normal" males in certain families and leaves an excess of abnormals.*

Families.	Sex Ratio.		Ratio of Males to 100 Females.	Difference.
	Males.	Females.		
All males hemophilic	413	337	122.55 ± 2.73	35.26 ± 3.39
Part males hemophilic	1,070	678	157.81 ± 2.02	10.4 × P.E.
All males color blind	114	100	114.00 ± 4.4	30.62 ± 6.52
Part males color blind	184	119	154.62 ± 4.83	4.6 × P.E.

Another characteristic of sex-linked lethal factors is, that in such forms as man a decreased proportion of female offspring should be produced in the matings in which no such excess of affected males exists. If the families in which *all* males are hemophilic or color blind, are contrasted as regards sex ratio, with those in which *part* of the males are abnormal and part normal a basis for comparison is afforded. Errors of classification, should they occur, should militate *against* finding a significant difference-

and can therefore be disregarded. When such a comparison is made the results are qualitatively similar and give strong support to the evidence already described.

In the cases of hemophilia the odds are enormously against the difference being due solely to chance (one to more than 2,000,000,000) and in the case of color blindness, in spite of the smaller numbers one to more than 500.

Statistical evidence, therefore, strongly indicates the presence of sex-linked lethal factors in man. More complete evidence can be obtained only after an intensive study of several generations of prolific families has been made. The theoretical and practical importance of making such studies is so great that a start on them should be made at the earliest opportunity.

54 (1636)

Acidosis from capillary poisons.

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In a study of pathological conditions of the capillaries, and the effects of these conditions on the body as a whole, we have sought for some agents which produce widespread capillary damage. There is a considerable number of substances classed as capillary poisons. Some of these, for example histamine, produce functional changes, others, such as uranium and diphtheria toxin, produce structural changes. If the change produced results in a damage to the capillary wall, there should be a decrease in its permeability. The tissue cells supplied by the capillary would receive less blood and oxygen, with a resulting abnormal metabolism. It seemed to us that this effect might take the direction of an acidosis. The following figures, obtained from experiments on dogs, the Van Slyke apparatus being used for the alkali reserve determination, bear out this conception.

A study of the table shows that uranium, cantharidin, arsenic, and diphtheria toxin, which cause widespread capillary damage, bring about a definite acidosis. Podophyllotoxin and emetine,

C.c. of CO_2 Bound as Bicarbonates by 100 c.c. of Plasma.[1] Normal.	Effect Produced.	Agent Used.
58.0	24.0	Uranium
54.0	30.0	Cantharidin
57.0	43.0	Arsenic
57.0	53.0	Podophyllotoxin
65.0	65.0	Emetine
58.0	72.0	Hydrazin
58.0	111.0	Histamine
47.0	41.0	Ether and histamine
56.0	18.0	Diphtheria toxin
53.0	25.0	Sodium nitrite
54.0	12.0	Potassium cyanide
64.0	64.0	Morphine
58.0	70.0	Double nephrectomy
50.0	30.0	Uranium after double nephrectomy
54.0	54.0	Emetine, jugular vein
54.0	54.0	Emetine, intestinal vein

[1] In the table the time relationships are omitted. In some instances, for example in the cyanide experiment, the duration of the poisoning was a few hours. In others the duration was a number of days.

whose action is confined to the intestinal capillaries, cause no acidosis. Hydrazin, which is not classed as a capillary poison, but which produces changes confined to the liver, and comparable to those seen in phosphorus poisoning, causes no acidosis. Histamine, given by subcutaneous injection in repeated and eventually fatal doses caused an increase in alkali reserve, given intravenously to an animal sensitized by ether, it causes acidosis.

If our conception of the cause of the acidosis from these capillary poisons is correct, it should follow that other agents which interfere with the supply of oxygen to the tissues in other ways would also cause acidosis. This is indeed the case. Nitrites, which induce methaemoglobin formation, and cyanides, which prevent the utilization of oxygen by the tissues, bring about a marked acidosis. To show that a depression of the nervous system and respiratory center is not a factor, we have given a dog 2 grams of morphine during a period of 6 hours, with no resulting acidosis. A double nephrectomy in itself causes no acidosis. Uranium given to an animal with both kidneys removed, induces the same degree of acidosis as in an animal with the kidneys intact. Again in poisoning with emetine, with marked effects in the intestinal capillaries, the blood from an intestinal vein is not different from that from the jugular vein.

We find therefore that marked damage to the liver and intestine fails to induce acidosis, and that the kidney also is not a necessary factor. We believe at present that the condition essential is an injury to the muscle capillaries.

55 (1637)

The glucose mobilization rate in hyperthyroidism.

By BERTRAM J. SANGER.

[*From the Medical Clinic, Presbyterian Hospital; Columbia University, New York City.*]

The work presented here is a preliminary report of some special investigations we have undertaken during the past twelve months at the Presbyterian Hospital in order to shed more light on the complex problem of hyperthyroidism. The decreased sugar tolerance, so frequently found in this condition, was taken as our point of departure. It occurred to us that the study of the respiratory quotient and the blood sugar at frequent intervals after glucose ingestion might give us a good deal of information as to how cases of Grave's disease utilize carbohydrate.

In brief, our procedure was as follows: the metabolism determinations were made with a 90-liter Tissot apparatus, using a Siebe-Gorman mask and Douglas valves. Samples of gas were taken over mercury in the usual way and were analyzed in duplicate in a Haldane gas analysis apparatus and in triplicate if the two analyses did not check satisfactorily. Previous to the use of the Haldane apparatus each day an analysis of outside air was made as a control. The usual technique was observed as to the preparation of the patient—14 to 16 hours fast and absolute rest for thirty to sixty minutes before the start of the determination.

After obtaining two basal periods each of ten minutes, blood was taken for a fasting blood sugar. The patient was then given a dose of glucose made as palatable as possible with a small amount of fruit juice. For the most part, a standard dose of 1.75 grams per kilo bodyweight was used. Twenty minutes after the glucose ingestion, the metabolic rate was determined, for a period of ten minutes if possible, and for the next two and a half hours, a determination was made each half hour. Blood was taken for blood sugar estimations 15 minutes, 60 minutes and 120 minutes post glucose. In all six normal controls and eight very definite cases of hyperthyroidism were studied.

The results were analyzed and plotted in the following way: with the fasting blood sugar, the fasting respiratory quotient and the basal metabolic rate taken as zero, the rise after the standard glucose dose was charted from this point, the blood sugar and respiratory quotient directly, and the metabolic rate as the percentage rise above that of the basal period.

In the first place, it was noted that the fasting respiratory quotient of hyperthyroids was somewhat lower on the average than that of the normals. The most striking finding, however, was the very rapid rise of the respiratory quotient in the hyperthyroid to a high level, approaching 1.0—a total carbohydrate respiratory quotient, some even going slightly above unity—and the maintenance of this high level, throughout the two and a half hours, while the normal controls rose more slowly, rarely reaching a figure much above .90 and tending to drop a little before the end of the experiment.

The typical blood sugar curve was found in all but one of the eight cases of Grave's disease studied. This patient was distinctly different from the others however. His metabolic rate was 53 per cent. above the normal, he had all the classical findings—positive eye signs, soft vascular struma, tremor, tachycardia and besides auricular fibrillation—yet in spite of all this, he was not losing weight and had no creatinuria on a creatin free diet. Glycosuria was likewise absent in this patient following the glucose tolerance test, though it was found in six out of seven of the other cases in this series. The respiratory quotient curve also did not fit in with the other curves obtained. Obviously his carbohydrate metabolism was not greatly deranged.

The comparison of the change in the metabolic rate between the normals and the hyperthyroids was very confusing. When plotted alike, the percentage rise above basal was much less in the cases of Grave's disease than in the controls. This may give an erroneous impression however, as it has been suggested that the abnormal cases should be figured on the basis of what the rise in calories would have amounted to had the patient's metabolic rate been normal. When figured in this way the two curves are very much alike. It seems likely therefore that the specific dynamic action of carbohydrates is much the same in both normal controls and cases of hyperthyroidism.

It seems fairly plausible from an analysis of the blood sugar and respiratory quotient curves that the hyperglycemia and low sugar tolerance so frequently found in Grave's disease are not due to any inability of the tissues to burn carbohydrate, but very probably to a decreased ability of the liver to store it. This is further substantiated by approximate calculations of the grams of carbohydrate burned. If 15 per cent. of the calories are assumed to have been derived from protein and the rest apportioned to carbohydrate and fat according to the respiratory quotient (table of Zunst and Schumburg) and the grams of carbohydrate metabolized, then figured from this, it will be seen that the increase in the amount of carbohydrate burned is very great in the hyperthyroids and very slight in the normals—that is, the normals are apparently storing carbohydrate while the hyperthyroids, stimulated by the carbohydrate plethora, are burning it. This fits in very well with the work of Cramer and his collaborators in England and Kuriyama in this country, who have found that thyroid fed animals (white mice and rabbits) on high carbohydrate diets showed only traces of glycogen in the liver, while control animals showed normal amounts. This would account for the low fasting respiratory quotient in thyroid disease, and would explain a clinical observation that has been noticed by some observers—namely, that it is very easy to produce acidosis in a hyperthyroid. One case in point, on the surgical side of the Presbyterian Hospital (History No. 30699), who was in for the treatment of hyperthyroidism and who had glycosuria on a regular diet, went into severe acidosis on being put on the standard strict diet (10 Carbohydrate – 100 Protein – 120 Fat) in an attempt to clear up the glucosuria.

In all the eight cases of Grave's disease studied, there was an original increase in the total metabolism of 30 per cent. or over;

in all but one case there was an increase in the carbohydrate metabolism as manifest by the high respiratory quotient and the calculated amount of carbohydrate burned. The plethora of carbohydrate in the blood stream might account for this stimulation of carbohydrate metabolism. All the evidence seems to point to the fact that the liver has a decreased ability to demobilize carbohydrate from the blood stream—or perhaps there is a hypermobilization rate.

56 (1638)

On a volatile sperm-stimulating substance derived from marine eggs.

By **G. H. A. CLOWES** and **E. BACHMAN.**

[*From the Research Laboratories of Eli Lilly and Company, Indianapolis, and the Marine Biological Laboratory, Woods Hole.*]

Sea urchins eggs suspended in sea water secrete a sperm-stimulating substance studied by Jacques Loeb and H. M. Fuchs, as well as the sperm-agglutinating substance investigated by Frank Lillie and O. Glaser. From preliminary experiments carried out last season at Woods Hole, it appears that this sperm-stimulating substance may be derived from the eggs of the sea urchin, star fish and sand dollar; that it is non-specific and is a comparatively simple volatile, organo substance, a product of enzymatic action or fermentation within the cell.

This substance, the exact constitution of which is not yet known, may be distilled from a neutral, acid or alkaline extract, the first distillate exerting an effect almost equal to that of the original extract. It is not destroyed by heating in a sealed tube in an autoclave for several hours, in a neutral or alkaline solution, but is weakened by heating with acid. It is very rapidly destroyed by iodine and other oxidizing agents, a brief exposure to N/5000 or N/10000 iodine solution causing a lowering in its stimulating activity of at least 100 to 1. This substance, when added to quiescent or attenuated sperm, greatly increases the facility with which the eggs are fertilized by the sperm.

A large number of simple volatile organo substances of the type of propyl, allyl and cinnamyl alcohol, were tested regarding their sperm-stimulating and fertilization promoting effects, and also their susceptibility to oxidizing agents and other chemicals, and were found to exhibit effects corresponding very closely with those exerted by the egg secretions and extracts. The exact constitution of the substance or substances present in the extract, has not yet been determined on account of the paucity of material at the end of the season, but it is proposed to continue this investigation next summer.

57 (1639)

On a method of producing chronic focal lesions in animals.

By **HANS ZINSSER** and **EDWARD H. RAYMOND, Jr.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, New York City.*]

Celloidin capsules are made by a method first used, we believe, by Dr. Clarke of the College of Physicians and Surgeons, though we are not sure of this, but certainly not entirely original with us. Small balls or globes of sugar in the form of some of the more commonly purchasable candies, are stuck to small silk threads with a hot forceps. These are dipped three or four times in celloidin, hardened for a short time in alcohol, and thrown into a jar with running water. The sugar diffuses in the course of ten or twelve hours, and a completely closed capsule is left. With a fine needle a hole is punctured through the capsule, the water drained out, and agar, inoculated with streptococci or other organisms desired, is injected into the capsule and allowed to harden. The puncture-hole is left open. The capsule is then dropped into the peritoneal cavity of a rabbit and the rabbit sewed up. In most cases the rabbits live for months. Some of them gradually emaciate, others will develop agglutinins. We have opened a number of rabbits from six weeks to four months after the capsule had been placed into them. In one case a rabbit into which the capsule had been placed in July was opened in the middle of November (over four months) and the capsule was found to contain living streptococci at this time. Apparently the organisms in the capsule are to some extent protected against phagocytes, and other protective factors. The capsules are usually surrounded by fibrin and strands of connective tissue, and lie in a membrane of tissue that has grown about them.

Incidentally, it has been noticed that in the case where the capsule had remained in place four months, the organisms were, at the end of this time, culturally and morphologically identical with the ones that had been put in, which furnishes some evidence, at least, against the mutations of streptococci in the animal body advocated by Rosenau.

58 (1640)

The effect of temperature and of hydrogen ion concentration upon the rate of destruction of antiscorbutic vitamine.

By **V. K. LAMER, H. L. CAMPBELL,** and **H. C. SHERMAN.**

[*From Columbia University, Department of Chemistry,
New York City.*]

Experiments were made upon 300 gram guinea pigs fed a new basal diet designed to furnish practically optimum amounts of all nutrients except the antiscorbutic vitamine. The latter was furnished exclusively in the form of filtered canned tomato juice. Relative amounts of this vitamin in the treated and untreated portions of this juice were measured by determining the amounts necessary to prevent scurvy or by a quantitative rating of the severity of the scurvy produced. The technique and the probable degree of precision of the results will be discussed in a later paper.

In the case of tomato juice of natural acidity, P_H 4.2, it was found that boiling for one hour destroyed practically 50 per cent., and for four hours practically 70 per cent. of the antiscorbutic vitamine present. The time curve of the destructive process is therefore much flatter than that of a unimolecular reaction. The latter finding applies also to similar heating experiments at 60° and at 80°. In such experiments at 60° to 100°, the temperature coefficients are relatively low ($Q_{10} = 1.1$ to 1.3).

In experiments in which the natural acidity was first neutralized in whole or in part, the juice then boiled for one hour and immediately cooled and reacidified, it was found at P_H 5.1 to 4.9 (natural acidity less than half neutralized) the destruction during one hour's boiling was increased to 58 per cent. Neutralization of a larger proportion of the natural acidity regularly increased the rate of destruction of the vitamine at 100°. When alkali was added to an initial P_H of 11, which fell to about 9 during the hour of heating, the destruction found by feeding of the juice thus treated but immediately cooled and reacidified, was about 65 per cent. On repetition of the last mentioned experiments but with reacidification omitted, and the treated juice stored up to five days in the refrigerator before feeding, the destruction found was 90 to 95 per cent. Whether the difference between the juices which were, and those which were not, reacidified is attributable wholly to the prolonged action of the hydroxyl ions at a temperature of 10° C., and P_H only 9, or whether there are here involved other factors possibly including a tendency toward reversal of the destructive process upon reacidification, remains to be determined.

59 (1641)

The tuberculin reaction and anaphylaxis as studied by the Dale method.

By **HANS ZINSSER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, New York City.*]

It is natural that the various tuberculin reactions, as well as other specific phenomena of hypersensitiveness in bacterial disease, such as the mallein and typhoidin reactions should have been thought of from the beginning as probably anaphylactic in nature.

We do not think it suitable in this preliminary communication to go into the details of the controversial literature that has been waged for some time concerning this problem. Our studies are not completed, but as far as they go, they show sharp results in that we have checked up skin sensitiveness in tuberculous and experimentally sensitized animals with the state of general anaphylaxis as indicated by the uterine reaction observed by the Dale method.

The uterine method used in this way gives more conclusive results than any other because it avoids the uncertainties that always attend general anaphylactic experiments carried out on guinea pigs with bacterial extracts.

Our results so far may be summarized as follows:

Tuberculous animals, unless inoculated with overwhelming doses, always become anaphylactic to tuberculo-protein. Positive uterine reaction is never obtained, however, before the end of the third week, and sometimes not until the sixth week at a time when the disease has made considerable progress.

Skin reactiveness may develop in such pigs, however, as early as the ninth day, long before the uterus gives any signs of general anaphylaxis.

There may, thus, exist in the tuberculous animal marked skin reactivity without any uterine hypersensitiveness.

Both skin reactiveness and general anaphylactic hypersensitiveness may fade together in the prelethal stages when the pigs are very sick.

Normal guinea pigs are easily rendered anaphylactic to extracts of tubercle bacilli by injecting them intraperitoneally on successive or alternate days, for ten injections, and testing them on and after the eighteenth day after the last injection.

Such anaphylactic pigs tested from the time of the last few injections until the time of the fully developed hypersensitiveness have never, except in two instances, given typical skin reactions, and in one of these two the reaction was not as marked as in the typical tuberculous animal, and in the other the possible absence of tubercles could not be excluded.

Guinea pigs sensitized experimentally with tuberculo-protein, therefore, may be very highly anaphylactic as evidenced by the uterus, and show absolutely no typical skin reaction.

There are two types of skin reaction, one which resembles the skin reaction obtained in human beings sensitive to horse serum, etc., which develops within a few minutes or within one half hour, appears to be chiefly a vascular reaction with edema, etc., and which fades without subsequent inflammation within a few hours. This has, occasionally, been observed in the anaphylactic pigs on intracutaneous injection, and is, we believe, probably a true anaphylactic skin reaction in all that this term implies. It should be noted, however, that as far as we know no careful analysis has ever been made to prove experimentally that such skin reactions run entirely parallel with general anaphylaxis.

The other type of skin reaction is the typical tuberculin, typhoidin, etc., reaction which begins gradually, within three, four or five hours, reaches its maximum after 24 or 48 hours, and does not fade for four or five days. It is marked by definite inflammatory reactions with perhaps some hemorrhage and a little necrosis. It is distinctly a cell-injury reaction.

Since this last reaction and general anaphylaxis plainly are shown not to go hand in hand, the question arises, are they of fundamentally different nature or are they perhaps reactions to two different substances in the tuberculin preparations. It has been suggested that the true tuberculin reaction is rather analogous to toxin hypersusceptibility than to true anaphylaxis, and is incited by hypersusceptibility to a toxic constituent of the tuberculin rather than to the tuberculo-protein.

Loewenstein and Pick have studied tuberculin chemically and believe that the substance which induces the typical tuberculin reactions belongs to the class of polypeptides and is dialyzable.

Working with fish bladder membranes we have been able to show, so far, that the substance in O. T. and in the alkalin extracts of ground tubercle bacilli which causes the skin reactions diffuses through such membranes.

Whether or not this dialyzate produces or fails to produce reaction with the sensitized uterus we have not yet been able conclusively to determine.

The general trend of our work, however, together with studies to be reported in another place, lead us to make the following preliminary suggestion:

Substances like whole proteins, cannot establish chemical or physical relationship with the body cells to any degree without the intervention of antibodies, because they are not diffusible. In the case of such substances, therefore, the antibody mechanism is necessary to establish such relationship.

The instantaneous nature in which the anaphylactic reactions which take place through the intervention of antibodies occur, suggests that these are cell surface phenomena.

Substances which have a smaller molecular structure than the whole proteins and are more diffusible, can react with cells without the intervention of antibodies. The determining criterion, therefore, upon which it depends whether a substance is antigenic or, in other words, an antibody former, is, therefore, its ability or inability to diffuse.

In the case of substances which can pass through membranes to some degree, antibody formation is not necessary, and hypersusceptibility may depend upon changes which cannot be measured as we can measure antibodies.

Also, because of the diffusible nature of these substances, the reactions may be intracellular and this would account for the later inflammatory reactions due to definite cell injury.

60 (1642)

A modification of Folin's uric acid method.

By **HENRY JACKSON, JR.** and **WALTER W. PALMER.**

[*From the Chemical Division, Medical Clinic, Johns Hopkins University and Hospital, Baltimore, Maryland.*]

In an effort to improve Folin's uric acid reagent it was found that by dialyzing under special conditions Folin's solution and evaporating the solution so dialyzed a superior reagent was obtained. A similar, though not identical, reagent was prepared by boiling down Folin's solution and filtering off the precipitate. When mixed in the proper proportions these two substances yield a reagent superior to Folin's in the following respects.

1. There is no precipitate such as is frequently encountered with Folin's solution.

2. The color developed with a given quantity of uric acid is about four and a half times as intense as that developed in Folin's method.

3. The color does not fade over a period of many hours.

Since this work was done we have learned of Wu's isolation of the pure ammonium phospho-18-tungstate.[1] This substance was prepared by his method and its chromogenic powers were found to be the same as those of our salt. Like the latter the pure ammonium salt gives a better color in the absence Na_2CO_3 and sulphite lessens the color markedly, facts which explain why Wu apparently did not recognize that his salt under proper conditions gave more color than Folin's original solution. Our method is simpler and yields more usable material than Wu's method would if used for the same purpose.

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[1] H. Wu, Jour. Biol. Chem. 1920, xliv, 189.

The actual method of blood analysis is somewhat modified. No sodium carbonate is used, the cyanide furnishing the requisite alkalinity. Benedict's standard must be used. The cyanide must be measured to an accuracy of 0.1 c.c.

ABSTRACTS OF THE COMMUNICATIONS,

PACIFIC COAST BRANCH.

Twenty-seventh meeting.

San Francisco, California, January 12, 1921.

61 (1643)

Colorimetric determination of hydrogen ion concentration by means of a double-wedge comparator.

By G. D. BARNETT and C. W. BARNETT.

[*From the Laboratories of the Division of Medicine, Stanford University Medical School.*]

In a former paper[2] a method was outlined for determining hydrogen ion concentration colorimetrically without the use of buffer solutions, and data for making such determinations for P_H values between 6.7 and 8.1 were given, using phenolsulphonephthalein as an indicator. The method consisted in the partition of a constant amount of indicator solution between pairs of test tubes of equal caliber, one tube of each pair containing 5 c.c. of weak acid, and the other tube 5 c.c. of weak base. When such pairs of tubes are viewed by transmitted light in the comparator of Hurwitz, Meyer and Ostenberg a series of colors is observed covering the range of the indicator, each color corresponding to a definite P_H value. A committee of the American Association of Bacteriologists later investigated and reported on the method,[1] and published a similar series of hydrogen ion exponents for brom thymol blue. More recently Gillespie[2] has extended the method to all of the indicators described by Clark and Lubs,[3] determining the P_H values of his tube pairs by comparison with buffer solutions whose hydrogen ion concentrations were checked by the gas chain method. The present work was in progress when Gillespie's paper appeared, and is largely a confirmation of his results.

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[2] Barnett, G. D. and Chapman, H. S., 1918, "Colorimetric determination of reaction of bacteriologic mediums and other fluids," *Jour. Amer. Med. Assoc.*, lxx, 1062.

Instead of dividing the indicator between two tubes, however, use has been made of a comparator consisting of a long narrow rectangular glass box containing a diagonal glass partition dividing it into two equal wedge-shaped compartments placed base to apex. One wedge is filled with acid indicator solution, and the other with alkaline indicator solution of the same concentration. Light transmitted horizontally through the box thus presents the complete range of color change of the indicator. For purposes of calibrating the color scale in terms of P_H, buffer solutions of known hydrogen ion concentration and containing the same indicator concentration were placed in a small glass box having the same fluid diameter as the large box. For any given buffer solution within the range of the indicator an exact color match is obtained. A scale along the lower edge of the comparator is divided into 100 parts and graduated from left to right. If the acid color of the indicator occupies the left end of the comparator, the readings of this scale will thus represent the percentage of alkaline indicator color present in the color blend observed at that point. The colors are best viewed against an oblique plate mirror reflecting the sky. The buffer solutions used were the phthalate, phosphate and borate mixtures of Clark and Lubs,[4] and their P_H values were checked with the hydrogen electrode, following the method of Clark,[1] and using the tables of Schmidt and Hoagland.

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[1] Conn, H. J., Harding, H. A., Kligler, I. J., Frost, W. D., Prucha, M. J., and Atkins, K. N., 1919, "Methods of pure culture study," *Jour. Bact.*, iv, No. 2, 107–132.

[2] Gillespie, Louis J., 1920, "Colorimetric determination of hydrogen ion concentration without buffer mixtures, with special reference to soils," *Soil Science*, ix, No. 2, 115–136.

[3] Clark, W. M. and Lubs, H. A., 1917, "The colorimetric determination of hydrogen ion concentration and its applications in bacteriology," *Jour. Bact.*, i.

[4] Clark, W. M. and Lubs, H. A., 1916, "Hydrogen electrode potentials of phthalate, phosphate and borate buffer mixtures," *Jour. Biol. Chem.*, 1916, xxv, 479–510.

The comparator was made in the laboratory from the glass of discarded X-ray plates jointed with balsam. Since only extremely weak acid and base have been used there have been no leakage difficulties. The boxes are carefully dried with filter paper after each day's use.

Dimensions: Inside length 35 cm.
Fluid diameter 15 mm.
Height 2.5 cm.

Gillespie has shown that the indicator transformations follow the law of mass action within the limits of error of the method, and has calculated "apparent dissociation constants" for each of his observations from the modified mass-law equation

$$K = P_H + \log \frac{x}{100 - x}$$

where $x/(100 - x)$ represents the partition ratio of the indicator in per cent. Similar values are given in the tables below. This constant is the P_H value of the mid-point of the indicator, *i.e.* the P_H value at which the indicator is half transformed from acid to salt form.

It will be noted that the constants above calculated show somewhat less deviation than those of Gillespie, probably because the method permits an exact color match, no interpolation being necessary. That our values are slightly higher than those of Gillespie is doubtless due to the fact that our measurements were made in the close neighborhood of 20°, instead of at the higher temperatures he used. The greatest discrepancies are with brom phenol blue and brom cresol purple, with which we have had some difficulty in obtaining a perfect color match.

In order to determine the P_H of unknown solutions we may construct a table or curve for each indicator, giving the theoretical value of the P_H for scale readings at convenient intervals. It is, however, more convenient to graduate the scale directly in P_H

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[1] Clark, William Mansfield, 1915, "A hydrogen electrode vessel," *Jour. Biol. Chem.*, 1915, xxiii, 475–486.

[2] Schmidt, Carl L. A. and Hoagland, D. R., 1919, "Table of P_H, H, and OH values," Univ. California Publications in Physiology, Vol. 5, No. 4, pp. 23–69.

TABLE OF DISSOCIATION CONSTANTS. (CALCULATED MID-POINT P_H.)

Indicator.	Solvent.	Percentage Indicator in Final Solution.	Scale[1] Reading.	P_H.	K.	Mean.
Brom phenol blue....	Alcohol	0.0004	27.1	3.52	3.95	
			41.4	3.72	3.85	
			52.8	3.94	3.89	
			67.2	4.16	3.85	
			78.7	4.40	3.83	3.87
Methyl red.........	Water	0.0004	30.0	4.65	5.02	
			38.5	4.82	5.02	
			50.0	5.03	5.03	
			58.6	5.19	5.04	
			61.4	5.22	5.02	
			64.3	5.26	5.00	
			72.9	5.41	4.98	
			75.6	5.50	4.99	
			81.5	5.65	5.00	5.01
Brom cresol purple...	Alcohol	0.0012	27.1	5.83	6.26	
			38.5	6.02	6.22	
			47.2	6.22	6.27	
			55.7	6.44	6.30	
			64.3	6.59	6.33	6.28
Brom thymol blue ...	Water	0.0004	24.2	6.59	7.09	
			35.7	6.83	7.09	
			47.2	7.03	7.08	
			55.7	7.19	7.09	
			67.1	7.37	7.06	
			75.6	7.56	7.06	
			81.5	7.75	7.09	7.08
Phenol red..........	Water	0.0004	18.5	7.12	7.77	
			30.0	7.37	7.74	
			38.5	7.56	7.76	
			49.0	7.74	7.77	
			58.6	7.93	7.78	
			70.0	8.16	7.79	
			81.5	8.43	7.78	7.77
Cresol red..........	Water	0.0004	30.0	7.74	8.11	
			35.7	7.91	8.17	
			47.2	8.15	8.10	
			58.6	8.28	8.13	
			64.3	8.39	8.13	
			71.5	8.52	8.12	8.13
Thymol blue........	Water	0.0004	15.7	8.10	8.83	
			18.5	8.17	8.83	
			30.0	8.44	8.87	
			35.7	8.54	8.80	
			44.3	8.78	8.89	
			58.6	9.01	8.86	
			75.6	9.43	8.93	8.86

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[1] The actual readings in this column were made on a centimeter scale, and are here converted to per cent.

intervals of 0.1 on each side of the mid-point. This may be done by giving to the quantity log $x/(100 - x)$ successive values from -0.9 to $+0.9$ in intervals of 0.1. From these equations corresponding values of x are readily obtained:

$\text{Log}\,\frac{x}{100-x}$ (P_H Difference).	x (Scale Reading).	$\text{Log}\,\frac{x}{100-x}$.	x
−0.9	11.2	+0.1	55.7
−0.8	13.7	+0.2	61.3
−0.7	16.6	+0.3	66.6
−0.6	20.1	+0.4	71.5
−0.5	24.0	+0.5	76.0
−0.4	28.5	+0.6	79.9
−0.3	33.4	+0.7	83.4
−0.2	38.7	+0.8	86.6
−0.1	44.3	+0.9	88.8
0.0	50.0		

If these values are indicated on the scale by the figures representing P_H differences, we may read the P_H value of an unknown solution by adding to the mid-P_H value (dissociation constant) of the indicator the differential quantity indicated by the scale reading obtained. Thus, if a scale-reading midway between +0.4 and +0.5 is obtained, using methyl red, the P_H of the solution will be 5.01+0.45, or 5.46. With reasonable care the error of such a reading is certainly not greater than 0.02 P_H, especially in the region of the mid-point, where the indicators are most used. Compensation for colored or turbid solutions is made by placing a small glass compartment of the same fluid diameter behind that portion of the comparator in which the match is to be obtained.

SUMMARY.

1. The method of determining hydrogen ion concentrations colorimetrically without the use of buffer solutions is extended to the group of indicators described by Clark and Lubs. Values of the dissociation constant at 20° of each of the indicators are given.

2. A double glass wedge comparator is described for making such determinations.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

One hundred thirteenth meeting.

College of the City of New York, New York City, February 16, 1921, President Calkins in the chair.

62 (1644)

The reserve energy of actively growing embryonic tissues.

By **MONTROSE T. BURROWS.**

[*From the Department of Surgery, Washington University Medical School, and the Research Laboratory, Barnard Free Skin and Cancer Hospital.*]

In 1902 Fletcher[1] showed that the sartorius muscle of a frog will give a maximum contraction when stimulated every five minutes over a period of two hours in an atmosphere of pure nitrogen. In an atmosphere of oxygen, the same muscle will contract for a much longer time and the fatigue developing in an atmosphere of nitrogen may be removed by placing the muscle again in an atmosphere of oxygen.

If differentiated muscle has sufficient reserve energy to allow it to contract for a period without oxygen, the question arises, will not the same reserve be found in an actively growing tissue.

In a previous article in 1917[2] the author described a method which allows one to study quantitatively the effect of various concentrations of oxygen on the growth of cells *in vitro*. In that paper the main point studied was the relative effect of pure oxygen and various dilutions of oxygen in nitrogen on the rate and extent of the growth. The tissues used were fragments of heart muscle and other tissue of chick-embryo of various ages. These experiments showed that the growth was practically the same in an atmosphere containing 8 per cent. of oxygen as in one containing a much larger proportion of this gas or in a pure oxygen atmosphere.

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[1] *J. of Phys.*, 1902, xxviii, 474.

[2] *Am. Jour. of Phys.*, 1917, xliii, 13.

A low concentration of oxygen beyond which no growth of cells will take place was also recorded. But as stated this was not accurately determined. There were certain discrepencies in the method of measurements and as must be stated here the tissues used for determining this point were fragments of the heart of 15, 16, and 18 day old chick-embryos.

In the present experiments, these objections to the technic used as outlined in the previous paper have been removed and tissues from embryos of various ages have been tested. The tissues chiefly used have been fragments of the heart muscle and body wall of 4 and 5 day old, 10 day old, and 15 day old chick-embryos.

One criticism of Fletcher's work is that he used a muscle of considerable thickness and in which the gaseous exchange must have been slow. I think, however, as Bayliss states, that this objection was obviated by his second experiment where he placed the fatigued muscle from the atmosphere of nitrogen into one of oxygen and noted a recovery from the fatigue.

I measured the distance oxygen diffuses readily into clots of blood, clots of plasma, and into tissue by means of the color changes in red cells. Into clots of chicken's blood the diffusion takes place readily only into a surface layer of 0.5 to 0.7 mm. in thickness. The hemoglobin in red cells lying below this layer or which are separated from the air by a layer of clotted plasma of that thickness becomes readily and permanently reduced. In this surface layer they maintain a bright red color.

In tissues the diffusion of oxygen may be more. It may exceed 3 mm. There are variations, however.

In all of these experiments fragments of tissue less than a mm. in diameter were used. They were planted in a layer of plasma 0.5 mm. or less in thickness. The culture chambers were made from one piece of glass tubing prepared according to the manner described in the previous papers (loc. cit.). The gas to be tested was passed through the chamber intermittently for from 1 to 2 hours. The capillary tube inlet and outlet were then sealed in the flame.

About the fragments of 4- and 5-day-old chick embryos in an atmosphere of pure nitrogen, the heart muscle cells were seen to grow a short distance out into the medium, and there was also an active growth and division of the serosa cells. A similar growth of cells was also seen about the fragments of the body wall. Dividing figures were seen in both types of cultures.

This growth commenced generally, however, only after a considerable latent period varying from 10 to 24 hours. It continued actively for only a few hours, when it ceased and an active rounding of the cell and fragmentation of the nucleus and the cytoplasm intervened.

In 7.48 per cent. oxygen or in air, this growth commenced always much earlier even after one or two hours. It continued actively for from 24 to 72 hours, when it slowly ceased. The cells did not disintegrate at once, but very slowly. Even after weeks or months, many have been found in a good state of preservation.

No growth was seen in the cultures of 10- or 15-day-old embryos in an atmosphere of pure nitrogen. A growth comparable to that of the younger embryos in pure nitrogen occurred about the fragments of 10-day chick embryos in an atmosphere containing 1.8 per cent. oxygen, and about the fragments of the 15-day-old chick embryos in an atmosphere of 5.4 per cent oxygen in nitrogen.

The results of these observations are graphically shown in the accompanying table and curve.

Heart muscle fragments of young chick embryos or the whole hearts will contract at once, when placed in the plasma of a tissue culture and warmed to the temperature peculiar to them. Fragments of older chick embryos and fetal chickens will not contract in the culture until a certain amount of growth and movement has taken place.

Fragments of the heart of 4- and 5-day-old chick embryos were found to contract actively in an atmosphere of pure nitrogen for as long as 5 or 6 hours. After this time the contractions became generally weaker, but often did not cease entirely for from 20 to 24 hours.

CONCLUSIONS.

1. These experiments with the contracting heart muscle fragments are in complete harmony with Fletcher's much earlier ones.

2. The cells of young embryonic tissue contain also a reserve energy. They may grow in an atmosphere of pure nitrogen for a short time. This absence of oxygen leads soon, however, to their rapid disintegration. Oxygen is evidently necessary for their preservation as Warburg conceived it.[1]

3. This ability for the cells to grow without oxygen is lost with development. It fails in the case of the cells of older embryos; as development proceeds, a greater and greater amount of oxygen is needed.

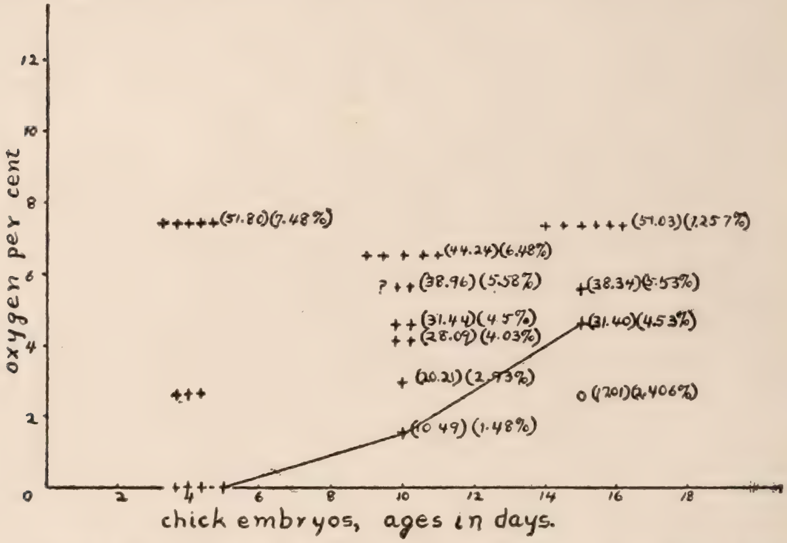

TABLE AND CURVE I. The Relative amount of growth in the cultures is indicated by the number of + signs. That noted in the 4 day old embryonic tissue in 7.48 per cent. oxygen is comparable to what is seen in air.

63 (1645)

Does growth require preformed carbohydrate in the diet?

By **THOMAS B. OSBORNE** and **LAFAYETTE B. MENDEL.**

[*From the Laboratory of the Connecticut Agricultural Experiment Station, and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

Carbohydrates are ordinarily regarded as indispensable components of the food intake. This belief is based on the presence of more or less carbohydrate in the food mixtures consumed by man and the higher animals, and the fact that sugar is a constant constituent of the blood. Furthermore, it has been concluded that carbohydrates are essential for the proper metabolism of the fats because ketone substances may be excreted in diabetes when sugar fails to be burned up in the normal manner in the organism.

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[1] *Ergeb. Phys.*, 1916, xiv.

We have found that *rats receiving a diet in which the amount of digestible carbohydrate was at most exceedingly small can grow from an early age to adult size.* The rations which we fed included protein—casein, edestin, or lean beef which had been thoroughly extracted with boiling water—inorganic salts, agar-agar, lard, butter fat and 0.4 gm. daily of dried brewery yeast furnishing vitamin B. The yeast can scarcely be regarded as a significant source of available carbohydrate. Success was likewise attained in experiments in which no agar-agar was introduced. In the latter case the only obvious sources of preformed carbohydrate were the yeast employed and such carbohydrate impurities as might still adhere to the protein preparation fed.

64 (1646)

Paramecium calkinsi sp. n.

By LORANDE LOSS WOODRUFF.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

There are at present four well-established species of *Paramecium* (*P. aurelia*, *P. caudatum*, *P. bursaria*, and *P. putrinum*) which fall naturally into two quite clearly defined groups. One group comprises *Paramecium aurelia* and *Paramecium caudatum* which are characterized by a relatively long spindle-shaped body. The other group includes *Paramecium bursaria* and *Paramecium putrinum* which exhibit a somewhat shorter and broader form, with a tendency toward a dorso-ventral flattening. All the species have a single micronucleus except *Paramecium aurelia*, which has two micronuclei each showing characteristic 'endosome' structure.[1]

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[1] H. S. Jennings and G. T. Hargitt: "Characteristics of the Diverse Races of Paramecium," *Journal of Morphology*, 1910, xxi, 495. L. L. Woodruff: "*Paramecium aurelia* and *Paramecium caudatum*," *Journal of Morphology*, 1911, xxii, 223.

The purpose of the present paper is to record the discovery in January, 1920, of a hitherto undescribed form of *Paramecium.* It has now been extensively studied in pedigree cultures for more than a year and during this time it has bred true. I therefore definitely designate it a new species, *Paramecium calkinsi,* in recognition of the fact that Professor G. N. Calkins of Columbia University introduced students of the Infusoria to exact, daily isolation, pedigree culture methods.

The general body form of the new species places it at once in the bursaria-putrinum group, but its micronuclei both in structure and number are identical with those of *Paramecium aurelia.* In brief, *Paramecium calkinsi* represents the '*aurelia*' type of micronuclear complex in the '*bursaria*' group of species.

Details of the structure and life history of *Paramecium calkinsi* will appear in the *Biological Bulletin.*

65 (1647)

The yeast test as a quantitative measure of vitamine.

By **WALTER H. EDDY, HATTIE L. HEFT, HELEN C. STEVENSON** and **RUTH JOHNSON.**

[*From the Department of Physiological Chemistry, Teachers College, Columbia University and the Department of Pathology, New York Hospital, N. Y. City.*]

The details of this paper will be reported in full in another publication. The experiments reported covered the following points:

1. A comparison of yeast test results (Funk[1] technique) with the material used by Osborne and Mendel[2] in a feeding test with rats. The test was shown to merely approximate the results of the feeding test but when the dilution of the extracts were such that the content of one gram was present in 250 c.c. of water the agreement was much more exact. This result agrees with Funk's findings that the extract test must fall within the steep part of the curve of stimulation if it is to be used comparatively.

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[1] Funk, C., and Dubin, H., *Journ. Biol. Chem.*, 1920, xliv, 487. Ibid., PROC. SOC. EXPER. BIOL. AND MED., 1920, xvii, 175.

[2] Osborne and Mendel, *Journ. Biol. Chem.*, 1920, xli, 451.

2. A report of the result of 284 determinations on varying dilutions of an extract of alfalfa meal which in its highest concentration contained the extract of 400 grams of meal in 1,000 c.c. of water. This shows that the curve of stimulation is a curve with a definite shape which rises steeply from the control point to an optimum and then gradually declines to the right of the optimum. It also shows that there is greater variability in the region to the right of the optimum in the individual determinations. These results indicate clearly that if vitamine B is a factor in the test it is only one of several and that critics of the test are right in saying that comparisons cannot be made between the extracts of equal weights of extracted material at the present itme.

3. A series of studies on other sources showing that dilution produces similar results to those in alfalfa extracts but that the optima and shape of the curve of stimulation differ with each substance tested though in general they show a steep rise, an optimum and in some cases a decline to the right of the optimum. These results confirm the conclusions of 2 and make clear that it will be necessary to find a basal diet for yeast which is optimum in all except vitamine before the results of tests can be made comparative. In the group of tests presented in this connection were included extracts of pancreas and other organs made with acidified and with neutral alcohol. The results fail to confirm the contentions of Swoboda[1] in regard to failure of pancreas to yield the test or to support his vitaminogen speculation.

4. Experiments are given to show that when the Medium F of Fulmer, Nelson and Sherwood[2] is substituted for Nageli solution in the alfalfa series the control values are markedly higher but that the alfalfa extract still exerts a stimulatory influence in every concentration used. These results fail to confirm their contention that the stimulatory effect is due purely to salt optimum and quality. They do indicate that the yeasts are extremely sensitive to such salt concentrations and that vitamine B is not the only factor in the test.

5. Experiments are presented to show the effect of alkali on the stimulatory power of the test. In every case we obtained a

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[1] Swoboda, F. K., *Journ. Biol. Chem.*, 1920, xliv, 531.

[2] Fulmer, Nelson and Sherwood, *Journ. Am. Chem. Soc.*, 1921, xliii, 186 and 191.

marked diminution of power by alkali treatment but never any where near so complete removal of the power as was obtained with adsorbent agents. With yeast autolysate the reduction was marked. With alfalfa extract it was not nearly so marked and in some concentrations the results justify Fulmer, Nelson and Sherwood's view that alkali does not affect the stimulus. Their conclusions would undoubtedly have been different had they tested enough concentrations to see the trend of the curve.

6. Experiments are given to further confirm the effect of known vitamine B precipitants and adsorbents upon the stimulatory power of extracts and a new reagent for use in this connection, viz., a carbon specially activated by Professor McKee, of the Department of chemical engineering of Columbia University, which was developed to adsorb basic substances. This substance has the power to remove the stimulatory power from an extract with the same efficiency as the Lloyd reagent, and when first washed free of all adherent matter with water can by boiling with glacial acetic acid be made to yield again its stimulatory material.

From these experiments it seems to follow that the test is not specific for Vitamine B as conducted by any of the methods now cited in the literature. On the other hand it seems also to follow that the vitamine B is not yet ruled out as one of the factors in the stimulation and to justify further investigation to secure an optimum medium for yeast comparable to those used in rat-feeding experiments.

66 (1648)

The antiscorbutic property of raw, dried and cooked apples and bananas.

By **MAURICE H. GIVENS, H. B. McCLUGAGE** and **E. G. VAN HORNE.**

[*From the Department of Physiology, University of Rochester, Rochester, N. Y.*]

Apples and bananas are the two most generally used of all the fruits consumed by man in this country. The use of these foods is advocated not only for healthy adults and infants[1] but indeed for the sick.[1] These two fruits have been classed for a long time as antiscorbutic agents. It is therefore important to determine as near quantitatively as possible their antiscorbutic potency in the raw state and after subjection to heat treatment such as is ordinarily employed in the preservation and cooking of these materials.

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[1] Pease, M. C., and Rose, A. R., *Am. J. Dis. Child.*, 1917, xiv, 379.

Experiments have been conducted on guinea pigs on a basal diet adequate in all respects except the antiscorbutic vitamine. To determine the presence or absence of this latter factor in raw, dried and cooked apples and bananas a daily allotment of these foods has been fed to the animals. We have found that a per diem dose of 10 grams of raw apples or of bananas will protect a guinea pig against scurvy for three months. On the contrary an equivalent amount of these foods cooked at 100° C. for fifteen minutes or dried at 55–60° C. (with the exception of apples which showed some antiscorbutic potency) or dried at 55–60° C. and cooked for fifteen minutes at 100 °C. will not protect the animals against scurvy.

67 (1649)

The determination of lung volume without forced breathing.

By **DONALD D. VAN SLYKE** and **CARL A. L. BINGER.**

[*From the Hospital of The Rockefeller Institute for Medical Research.*]

The dilution method for determining lung volume, invented by Davy and modified by Bohr and his coworkers, and recently by Lundsgaard and Van Slyke, rests on the principle of mixing the air in the lungs with a known volume of foreign gas (H_2 or O_2), and calculating the air content of the lungs from the extent to which either the foreign gas or the nitrogen of the lung air (Lundsgaard and Van Slyke) is diluted. This method yields satisfactory results when the subject can breathe deeply, so that 4 or 5 respirations cause complete mixture of the lung air with the diluting gas. When the subject, however, because of weakness or respiratory disturbance, cannot greatly increase the depth of his respirations, so much time is required for complete mixture that volume changes due chiefly to absorption of oxygen make accurate results unobtainable.

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[1] Myers, V. C., and Rose, A. R., *J. A. M. A.*, 1917, lxviii, 1022.

We have been able to avoid this difficulty by modifying the principle, and basing the calculation not upon the dilution of one gas, but upon the volume ratio of two gases. The subject breathes to and from a bag containing oxygen, sufficient to satisfy his requirements for the length of the experiment, and mixed with the oxygen a known volume of hydrogen approximating the volume of the nitrogen in the lungs. The CO_2 is removed by a scrubber of sodium hydroxide shells. After mixture is complete, the N_2 and H_2 in the gas are determined. Since

$$\frac{\text{initial vol. } N_2 \text{ in lung air}}{\text{initial vol. } H_2 \text{ in bag}} = \frac{\text{final per cent. } N_2 \text{ in gas mixture}}{\text{final per cent. } H_2 \text{ in gas mixture}},$$

$\text{vol. } N_2 = \text{vol. } H_2 \times \frac{\text{per cent. } N_2}{\text{per cent. } H_2}$. Since the nitrogen constitutes 0.791 of the air, the volume of air in the lungs is $\frac{\text{vol. } N_2}{0.791}$. The air volume thus determined is corrected by subtracting air present in the dead space of the apparatus at the start, also any air present as impurity in the oxygen or hydrogen used. Changes in volume of oxygen or CO_2 in the system do not affect the results, and absorption of hydrogen is negligible. Without changing the respiration from that normal at rest, results for residual air are obtained after about 2 minutes breathing that are not altered by further breathing and that agree with the results obtained by the Lundsgaard-Van Slyke method in subjects capable of properly cooperating in the latter. The error of the present method does not appear to exceed 4 per cent. of the lung volume estimated.

For the safe use of hydrogen two precautions are essential: The hydrogen, especially if made by the action of acid on a metal, such as zinc, should be tested for arsine to avoid fatal poisoning; and flames should be kept away from the apparatus to prevent explosion.

68 (1650)

The destructive effect of oxidation on antiscorbutic vitamine.

By **ALFRED F. HESS** and **LESTER J. UNGER.**

[*From the Department of Pathology, College of Physicians and Surgeons, New York City.*]

As stated elsewhere,[1] the antiscorbutic vitamine is destroyed by oxidation. Such was found to be the case when 4 c.c. of a normal solution of hydrogen peroxide were added to a liter of raw milk, which was then placed in the incubator overnight. Bacteria did not develop in the incubator under these conditions. When 80 c.c. per capita of this milk was fed to guinea-pigs, in addition to oats, they all developed scurvy in about three to four weeks, a result similar to feeding experiments with milk which had been autoclaved for one hour at 120° C. The addition of orange juice to the dietary served either to protect or to cure animals on this dietary.

Orange juice subjected to oxygen for a short period was likewise found to have lost some of its potency. Previously we had found that milk or tomato juice which had been shaken had lost some of this vitamine. Probably this deleterious action is partly due to the effect of oxidation. The harmful effect of "aging" may also be interpreted in this way.

As foodstuffs undergo oxidative processes frequently in the course of various manipulations, no doubt this factor plays an important rôle. This action probably explains the differences in the antiscorbutic potency of foodstuffs which have been treated in apparently similar ways, for example, of milk which has been heated in open pans or in hermetically sealed bottles.

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[1] Hess, A. F., *Brit. Med. Jour.*, 1920, ii, 147.

69 (1651)

The influence of venous return and arterial resistance on the pressures within the right and left ventricles.

By CARL J. WIGGERS.

[*From the Physiological Laboratory, Western Reserve University Medical School, Cleveland, Ohio.*]

I. ARGUMENT.

The question, is the response of the ventricle, under conditions of equal irritability, fundamentally determined by the initial length of its muscle fibers or by the initial tension exerted upon them, is of general physiological importance. The related question, can variations in initial volume (*i.e.*, initial length) occur independently of changes in initial tension, in the mammalian heart, is of far-reaching clinical interest as well. As regards the second question, the experimental results of Frank[1] and those of Straub[2] supply an answer which is contradictory to that of Patterson, Piper and Starling[3]. The latter investigators believe to have demonstrated that initial length alone determines the magnitude of the cardiac response, irrespective of whether initial tension is simultaneously altered in the same or reverse direction. Gesell[4] holds that both factors may be concerned but seems inclined to believe that changes in initial length play their important rôle when ventricular filling is relatively small.

While the fact can not be denied that the bulk of evidence apparently points to the conclusion that initial length fundamentally determines the magnitude of contraction in skeletal muscles, it is not so clear how such changes can *promptly* adjust the work of the heart to sudden changes in venous inflow or arterial resistance—except, in so far as these length changes are primarily due to changes in initial tension. This thought is suggested by the following premises: The diastolic volumes of the ventricles can be increased beyond their normal capacity either (*a*) by an increased initial pressure overcoming the inherent tendency of the ventricles to resist stretching, or (*b*) by a reduction of this inherent power of the ventricle to resist stretching, *i.e.*, by a reduction of tonus.[1]

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[1] Frank, *Ztschr. f. Biol.*, 1895, xxxii, 370.

[2] Straub, *Deut. Arch. f. klin. Med.*, 1914, cxv, 531; 1914, cxvi, 409.

[3] Patterson, Piper and Starling, *Jour. Physiol.*, 1914, xlviii, 465.

[4] Gesell, *Am. Jour. Physiol.*, 1916, xxxix, 239; 1916, xl, 267.

According to the hypothesis of Patterson, Piper and Starling it would be necessary to assume that an augmented venous return, for example, causes a prompt reduction of tonus. Our entire experience teaches us, however, that the degree of tonus in a muscle of any type is not capable of being rapidly changed; on the contrary, such changes occur very slowly. The results of Patterson, Piper and Starling, corroborated in my own work, indicate, however, so prompt a response on the part of the ventricle, as to make this almost presumptive evidence against the view that tonus changes are primarily or chiefly concerned. To supply quite certain proof that such changes are, on the other hand, associated with and probably due to simultaneous changes in initial tension requires a careful study of the pressure changes in the right and left ventricles during the early response of the right heart to changes in venous inflow and arterial resistance.

II. EXPERIMENTAL RESULTS.

The venous inflow into the right heart of intact animals was increased by allowing a graded inflow of normal saline into the jugular vein. Increased arterial resistance was produced, in experiments here reported, by partial compression of the thoracic aorta. Records of right and left intraventricular pressures were synchronously recorded by optical manometers. The results of 17 such experiments may be briefly summarized:

1. *Effects of Saline Infusion.*—Beginning with the very first beat when the initial (diastolic) volume of the heart increases, after such saline infusion has started, the initial tension in the

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[1] To prevent misunderstanding, it should be noted that I define the term "tonus" as that partial state of contraction which persists during diastolic rest, and by virtue of which muscle resists stretching. The statement of Patterson, Piper and Starling, that tonus is "synonymous with the physiological condition or fitness of the muscle and its measure is the energy set free per unit length of muscle fiber at each contraction of the heart," I believe, expresses the end effects of tonus changes but does not describe the nature of the tonus phenomenon itself.

right ventricle is at once elevated. As the heart continues to dilate during diastole, this elevation of pressure increases more and more. After the second beat the pressure-maximum is also increased in the right ventricle. Two to three beats are usually required before the initial and maximum pressures in the left ventricle are similarly altered. Systolic discharge and the intraventricular pressure-maximum continue to increase only so long as initial tension also continues to increase. In these cases, increased diastolic distention is, therefore, never dissociated from increased initial tension. Tonus changes may simultaneously operate to lengthen the muscle fibers independent of initial tension but, if so, their effects are entirely obscured. If this condition of increased inflow persists in a stationary manner, for a matter of 15 to 20 minutes, however, it may happen that then the heart dilates further, even while the initial pressures in the right and left ventricles decline. Such a dilatation, evidently due to a decrease in tonus, is always accompanied by a reduction in systolic discharge and in the pressure-maximum in both ventricles.

2. If arterial resistance is suddenly elevated during partial compression of the thoracic aorta, the systolic discharge is decreased for a few beats (usually 2–3) resulting, as also shown in Patterson, Piper and Starling's results, in a diastolic distention and increased initial length. Systolic discharge returns to normal about the fourth or fifth beat. Careful study shows that the pressure-maximum is elevated at once in the left ventricle, and by the third beat the initial pressure is also measurably although but slightly increased. At the fourth or fifth beat where the systolic discharge returns to normal there is a significant increase in the initial pressure in the left ventricle also. Then, for the first time, initial tension and pressure-maximum in the right ventricle also increase.

These results favor the conclusions, (1) that initial tension changes are apparently always associated with changes in initial length resulting from alterations in venous inflow or arterial resistance; (2) that in the intact animal, changes in initial tension play the predominant rôle in determining the response of the mammalian ventricle.

70 (1652)

Concerning anaphylaxis following the administration of diphtheria antitoxin.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School.*]

Sensitiveness of human beings to horse protein is fairly widespread as indicated by numerous reports of cases of serum sickness following administration of various therapeutic sera. The impression is, however, that in diphtheria the danger from this source is particularly slight. This comparative freedom of complications of anaphylactic nature following the administration of diphtheria antitoxin in emergency during the War has led to abandoning the preliminary skin test for sensitiveness in certain medical units.[1]

We attempted to approach experimentally this question of apparent tolerance to anaphylaxis during diphtheria intoxication. We have observed that sensitized guinea pigs receiving subcutaneously large excess of diphtheria toxin withstand the intravenous injection of at least five lethal doses of the antigen to which they were previously sensitized. This apparent resistance appears about 12 to 14 hours after the administration of toxin and just about the time when the outward symptoms of intoxication begin to manifest themselves.

With the view of eliciting the mechanism of this phenomenon we have made thus far the following observations:

The antitryptic titer of the blood of guinea pigs injected with the toxin does not appreciably deviate from normal up to the time of death. The mechanism regulating the antitryptic titer of the blood remains unimpaired in these animals, however, since an injection of antigen to which they are sensitized is followed by a typical rise of antitrypsin. This rise of antitrypsin, incidentally can be interpreted as indication that the humoral phase of the anaphylactic response of the animals is not abolished by the previous injection of toxin.

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[1] Personal communication.

If the same dose of toxin is overneutralized with antitoxin in vitro before injection, it does not protect the sensitized guinea pigs from immediate death when even a single minimal lethal dose of antigen is introduced intravenously. On the contrary the same dose of toxin heated for thirty minutes at 80° C. protected guinea pigs from anaphylactic shock just as unheated toxin did. Heating of the toxin for 30 minutes at 100° C., however, destroys this property of toxin even if much a larger amount of such toxin is injected.

Since the culture medium containing toxin contains also 1 per cent. peptone, a control sensitized guinea pig, instead of toxin received peptone in the amount ten times that present in culture medium carrying the toxin. This guinea pig died immediately after the intravenous injection of antigen, thus showing no protection. It seems thus that the clinical observation concerning apparent diminution of anaphylactic reactivity during diphtheria intoxication is borne out by this preliminary experiment.

71 (1653)

Studies in the physiology of vitamins: Is water soluble vitamin identical with secretin?

By GEORGE R. COWGILL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

A similarity in the physiological effects of vitamin B and substances which promote secretion has been alleged by several investigators.[1] We have examined a number of solutions such as extracts of rice polish, wheat embryo, navy bean and yeast, and neutralized tomato juice, demonstrated to contain vitamin B, for their possible action on the secretory function of the pancreas and liver. The products used were tested for vitamin B content on polyneuritic pigeons, and on dogs which had lost their appetite for several days after having been fed a diet lacking this dietary essential. Vitamin B has been shown to restore appetite in such animals.[1] The effect of the products on the flow of pancreatic juice and bile was noted in anesthetized dogs in which the pylorus was ligated to prevent secretion due to discharge of acid chyme from the stomach, and the gall bladder bile was prevented from discharging by ligation of the cystic duct. Normal dogs and dogs fed a diet lacking vitamin B were used. It is planned to experiment upon polyneuritic dogs as well. Fresh secretin solutions prepared by the usual method were injected as a control.

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[1] Voegtlin and Myers: *Jour. Pharm. Exper. Therap.*, 1919, xiii, 301. Uhlmann; *Zeitsch. f. Biol.*, 1918, lxviii, 3.

Except in the case of tomato juice, all of these products gave negative results. The secretin solutions, however, in comparatively small amounts always produced a characteristic and vigorous flow.

72 (1654)

Theoretical considerations bearing upon the chemotherapy of arsenical compounds.

By **GEORGE W. RAIZISS, JAY F. SCHAMBERG** and **JOHN A. KOLMER.**

[*From the Dermatological Research Laboratories, Philadelphia, Pa.*]

Chemotherapy is essentially the study of the toxic and therapeutic properties of chemical compounds. Its main purpose is to establish the maximum tolerated and minimum curative doses. The numerical value representing the ratio of these two doses is the chemotherapeutic index. Chemical compounds possessing the highest chemotherapeutic indices in experimental infection are usually the best adapted for the treatment of disease in man.

Ehrlich, Bertheim and Hata[2] were the first to engage in systematic chemotherapeutic work, in the course of which numerous new chemical bodies were synthesized. They were all derivatives of one certain compound called atoxyl, selected because it was the only organic arsenical known at that time which possessed trypanocidal properties, although to a very small degree. The changes in the chemical constitution of atoxyl led finally to the elaboration of a derivative many times more powerful in the cure of protozoön infection. At present, a considerable amount of work is being performed along the lines established by Ehrlich and numerous organic arsenicals have been prepared, still, the synthesis of new chemical compounds merely for the purpose of testing their toxic and therapeutic properties and thereby finding the best drug for the treatment of disease will not advance the science of chemotherapy beyond mere empiricism.

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[1] Karr: *Jour. Biol. Chem.*, 1920, xliv, 255.

[2] Ehrlich, P., and Hata, S. "Die Experimentelle Chemetherapie der Spirillesen."

Is it impossible to find a relationship between certain atomic groupings and toxicity or curative effect? Once the influence of these various groups upon the parasite or the animal body is appraised the synthesis of new compounds will assume a more rational course. Some very valuable findings have already been made in this direction by Ehrlich who discovered the remarkable trypanocidal and spirillocidal effects of the arseno group ($As = As$) when attached to the nuclear carbon atom.

Our attention has been attracted to the influence of the amino group upon toxicity and therapeutic effect when present in organic arseno compounds. We confined our study to the derivatives of 3, 3′ diamino –4, 4′ dihydroxy arsenobenzene, the dihydrochloride of which is the important remedy in syphilis known as salvarsan or arsphenamine. When this substance is converted into other compounds containing various substituents in the amino group, the toxicity and curative properties are influenced to such an extent as to lead to the assumption that the amino group is as vitally essential as the arseno group. As an illustration, let us consider a condensation product of arsphenamine with sodium formaldehyde sulphoxylate known as neoarsphenamine. As described in the patent papers and also in the work of Raiziss and Falkov,[1] it is evident that substitution took place completely in one amino group and partially in the second. The work of Schamberg, Kolmer and Raiziss[2] and also Roth[3] show that the average maximum tolerated dose of this compound is 0.254 milligrams per kilo of body weight, whereas that of arsphenamine is 100 milligrams. Considering the arsenic contents of these two substances, 50 milligrams of arsenic in the form of neoarsphenamine is tolerated as compared to 30 milligrams in the form of arsphenamine. Here it is evident that a change in the amino group resulted in the reduction of the toxicity amounting to about 40 per cent. The minimum therapeutic dose, however, was increased from 23 milligrams to 40 milligrams or in terms of arsenic from 6.9 milligrams to 8.0 milligram, *i.e.*, the curative power decreased 16 per cent. In the 3, 5:3′, 5′—tetraamino 4, 4′—dihydroxyarsenobenzene[1] we have a derivative which contains two more amino groups than arsphenamine. The maximum tolerated dose established by us is equal to seventy milligrams per kilo or in terms of arsenic 18.2 milligrams, an increase in toxicity of 39 per cent. The therapeutic effect remains as with arsphenamine. We also prepared 4, 4′—dihydroxyarsenobenzene—3, 3′—diaminoacetic acid, 4, 4′—dihydroxyarsenobenzene—3, 3′—alpha diaminopropionic acid, 4, 4′—dihydroxyarsenobenzene—3, 3′ alpha diaminobutyric acid, 4, 4′—dihydroxyarsenobenzene—3, 3′ alpha diaminovaleric acid. All of the above compounds are derivatives of arsphenamine in which both amino groups contain fatty acid substituents. In each case we observed a considerable reduction in the toxicity as well as therapeutic effect. We may conclude, therefore, that the toxicity as well as the curative power of organic arseno compounds is dependent to a considerable extent upon the fact as to whether the amino groups are free or substituted.

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[1] *Journal Biol. Chem.*, March, 1921.

[2] Schamberg, J. F., Kolmer, J. A., and Raiziss, G. W., *Amer. Jour. of Med. Sci.*, clx, No. 188.

[3] Roth, G. B., *Archiv. Dermat. and Syph.*, II, No. 3, 301.

73 (1655)

Refractometric studies with the sera of syphilitic patients under arsphenamin and neo-arsphenamin treatment.

By **KEIICHI TOKUDA.**

[*From the Dermatological Research Laboratories and the Wistar Institute of Anatomy and Biology, Philadelphia, Pa.*]

Refractometric studies were made by the writer upon thirty-two cases of *untreated* syphilis and the following observations were made:

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[1] Raiziss, G. W., and Gavron, J. L., *Jour. Am. Chem. Soc.*

1. There is a marked increase in the refractive index of the serum and also in the globulins in Syphilis, *especially in active secondary cases*. This confirms the findings of Rowe.

2. The refractive index of the serum is highest in secondary cases, lowest in the congenital and is intermediate between these two in the tertiary cases. The figures for total proteins, albumins, globulins and the relative amount of globulin are somewhat higher in secondary than in tertiary syphilis, the figures for congenital syphilis being somewhat lower than those of the latter.

3. Considered in relation to the Wassermann reaction of the sera, before treatment, the strongly positive cases show values of total proteins, albumins, globulins and relative amount of globulins *higher*, and values of non-proteins correspondingly lower than the weakly positive cases.

4. During a course of fourteen intravenous injections of arsphenamine (0.4 to 0.6 gm.) and neoarsphenamine (0.9 gm.), each drug being given at weekly and semi-weekly intervals, the refractometric studies (made before each injection) show the following results:

5. Classified according to the Wassermann reaction of the sera *before treatment*, there are no sufficiently constant or striking differences to warrant differentiating between the strongly and weakly positive series.

6. Considered according to the *intervals* of injection the data have much significance. The refractive indices of the sera, when *neoarsphenamine* was given at seven-day intervals, show a more uniform and continuous decline during the fourteen weeks of treatment, than when arsphenamine was similarly injected. *The relative amounts of globulins*, on the other hand, show more rapid decline during weekly arsphenamin than neoarsphenamin injections.

During semi-weekly periods of administration the changes are very irregular. The relative amounts of globulins, however, behave as during the weekly periods.

7. Classified according to the *degree of resistance* of the patients to anti-syphilitic treatment (as indicated by repeated Wassermann tests) the following was observed:

When the Wassermann reaction remained persistently positive, the refractive index, the percentage of total proteins and the relative amount of globulins of the sera showed little or no tendency to drop below their original values. When the Wassermann reaction, on the other hand, became very readily negative the curves fell progressively and fairly rapidly during the course of injections.

The writer is indebted to Dr. John A. Kolmer and Dr. Jay F. Schamberg for the laboratory and clinical facilities of the Dermatological Research Laboratories and the Polyclinic Hospital which they kindly placed at his disposal. Special acknowledgement is due to Dr. Charles Weiss of the D. R. Laboratories who compiled and interpreted the data and prepared this manuscript for publication.

74 (1656)

The analysis of factors which determine the life and growth of transplanted tissues.

By LEO LOEB.

[*From the Department of Comparative Pathology, Washington University School of Medicine, St. Louis.*]

1. We may distinguish between two sets of factors determining the fate of transplants: (*a*) primary or constitutional; (*b*) secondary or extraneous factors. The former comprise the individuality and species differentials, and possibly organ specific factors; the latter include such factors as age, sex, pregnancy, infection and immunity. There are in addition general factors as oxygen supply, character of circulation and temperature; the latter we shall at present leave out of consideration.

If we take the subcutaneous transplantation of the thyroid gland as type, we find the following variables which may be influenced by the two sets of conditions: (*a*) the amount of surviving parenchyma and its growth energy; (*b*) the behavior of the connective tissue cells; (*c*) of blood and lymph vessels, and (*d*) of the lymphocytes of the host towards the transplant.

2. In a series of earlier papers we have analysed the effect of the individuality and species differentials on these four variables

and we found a gradation in the effects observed which was in accordance with the relationship between host and graft.

3. Tyzzer and others, and especially Murphy and his collaborators, have shown that the appearance of lymphocytes in the case of transplanted tumors is an index of immunity against these tumors. Murphy and Rous showed that such an immunity cannot be produced in chick embryos, even in the case of hetero tumors, and Bullock and Rohdenburg found the same in newly born rats.

An analysis which we undertook recently of the results of transplantation carried out by numerous investigators in various classes of animals very strongly suggests the conclusion that the individuality (homoio) reaction is usually absent in embryonic and adult invertebrates and in embryonic forms of vertebrates. The species or class reaction (heteroreaction) is present, but less pronounced than in higher forms. In lower adult vertebrates the individuality (homoio) reaction is definitely found; but there are some indications that it is as yet not so pronounced as in higher vertebrates. As far as we can judge from experiments carried out for other purposes, we may therefore conclude that an ontogenetic and phylogenetic evolution of the individuality reaction has taken place and that the immunity reaction is probably dependent upon a special application of this evolution of the species and individuality differentials.

4. We have previously stated that in guinea pigs the individuality reaction is found in animals of various age, sex, and in pregnant animals.

For some time past we have been carrying on more extensive studies on the influence of these secondary factors in guinea pigs. We varied the age of the host as well as of the donor in order to study the effect of age on the four variables mentioned above. Thus far we can state that the lymphocytic reaction may be very marked not only if the tissue is transplanted into adults, but even if it is transplanted into guinea pigs a few days old.

The individuality reaction exists in pregnant as well as in nonpregnant guinea pigs. Autotransplantation of the thyroid gland in pregnant animals led in some cases to abortion. This did not noticeably influence the individuality reaction. Pregnancy did not improve the growth of the transplanted thyroid; on the contrary in some cases it seemed to diminish it. This was perhaps due to secondary factors (pressure of the distended skin which injures the graft or makes its sterile introduction into the wound more difficult); in a number of cases the growth of the transplant was equally good in pregnant and non-pregnant animals.

Localized infection of the graft with ordinary bacteria does not call forth a lymphocytic reaction in case of autotransplantation and it does not prevent its appearance in the case of homoiotransplantation. It may, however, interfere with the other variable factors and call forth a greater production of fibrous tissue. This may be associated with retardation in the organization of the necrotic center and with partial destruction of the parenchyma. Sex does not influence the four variables in the case of thyroid transplantation in the guinea pig. Whether the inferiority in results obtained after transplantation from child to mother which we found previously, is due to the action of a constitutional or of an extraneous factor remains still to be determined.

75 (1657)

The vasomotor response in anemia of the medulla oblongata:

(1) The splanchnic vaso-constrictor fibers.

(2) The relation of the splanchnic constrictor fibers to the secretion of adrenalin.

By CORA S. WINKIN.

[*From the Department of Physiology of Columbia University.*]

The experiments here reported deal with a series of studies on the analysis of the vasomotor response in asphyxia. The first of this series had particular reference to the part played by the splanchnic constrictor fibers in the response. The second series was concerned with the relation of the activity of these fibers to the secretion of adrenalin. The procedure throughout was the infliction of a complete but temporary anemia on the entire brain, according to the technique of Stewart et al.,[1] by clamping off the arterial circulation of the head. Occlusion in these experiments was used essentially as a constant and powerful stimulus for setting off a vasomotor response which was known to be aroused by the medullary centers. The experiments were all done on cats. Certain additional controls had to be obtained for the work on the adrenals which will be described below. These experiments were undertaken in connection with the studies on the functional organization of the nervous system carried on by Professor F. H. Pike and his collaborators.

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[1] Stewart, Guthrie, Burns and Pike, *Journ. Exp. Med.*, 1906, viii, 289.

THE SPLANCHNIC EFFECT

The evidence on the particular nervous channels involved in the vasomotor response to asphyxia was obtained by testing the response after the infliction of given nervous lesions. The response was found to depend almost entirely on the constrictor fibers of the splanchnic nerves. No lesion of the extrinsic cardiac nerves significantly altered the response to occlusion. Injection of curare, with the elimination of the pressor effect produced indirectly by the skeletal innervation also did not modify the response appreciably. However, section of the splanchnic nerves immediately below the diaphragm abolished all vasomotor response to asphyxia.

It was possible to work out the pathway of the splanchnic fibers with more precision. Section of the spinal cord in the upper thoracic region was found to give the same effect as section of the splanchnic in the region of the diaphragm; it also achieved a complete interference with the vasomotor response on occlusion. No other lesion within the splanchnic outflow, however, removed the response. Section in the upper lengths of the sympathetic chain, or section of the cord in any segment below the upper thoracic, allowed the response to persist with only partial diminution. Since the two lesions which abolish completely the asphyxial response are such as must definitely intercept all impulses from the brain to the periphery, it seems that the splanchnic outflow is normally in intimate functional continuity with the brain, and that all impulses for its release must arise physiologically within the medulla. The inability to abolish the response completely by sections in the sympathetic chain only a segment or two above the diaphragm or in the cord below the level at which the highest fibers to the splanchnics leave the cord, argues for the existence of a double functional pathway outside and within the cord down which constrictor impulses may travel. The level at which the highest fibers leave the cord is somewhat higher than that given by the anatomical investigations of Langley, Ranson, etc. While it varies from animal to animal it may run as high as the second or third thoracic.

THE EFFECT OF ADRENALIN

The work on adrenalin was preceded by a series of control experiments in which the effect on the vasomotor response of repeated occlusion in the same animal was worked out. It was found that in intact animals, or in animals in which lesion of the cardiac nerves had been inflicted, the constrictor effect could be obtained practically indefinitely, the animal responding as often as fifteen or twenty times in succession. Whereas neither the intensity nor the time occupied by the response was greatly affected, the contour of the blood pressure curve showed a considerable change as the number of occlusions was increased. Beginning with about the eighth or tenth occlusion, the curve was found to dissociate into two essentially distinct constrictor effects, each occupying about one half the time of the total effect.

It was found that on tying off the adrenal glands in other animals, no such number of repeated occlusions could be obtained. These cats failed before the tenth occlusion, often much earlier. Furthermore, after one or two responses following the tying off of the glands, the response was much shortened and soon came to occupy only about half the time of the normal response, or less. It seemed to approximate the first half of the dissociated curve of the exhausted, but otherwise anatomically intact animals.

According to these data, adrenalin is involved in a double relation to the splanchnic constrictor fibers. In the first place, its increased secretion, which is thrown out after a considerable latent period into the blood stream, is the agency that makes possible the maintenance of the effect initiated by the splanchnic fibers. These experiments seem therefore to demonstrate the adjuvant nature of the emergency action of adrenalin long postulated by Cannon.[1] In the second place, however, the existence of adrenalin in the blood stream is apparently necessary for the maintenance of vasomotor tone itself. The rapid exhaustion of the available supply of adrenalin in the blood stream, obtainable in these experiments appears the factor responsible for the early breakdown of the vasomotor system. Elliott[2] has argued for such a function of adrenalin from evidence of a different character.

Finally the entire evidence of these studies points to a complete dependence on the functional conductivity in the brain stem of both the initiation of the vasomotor effects by the splanchnics, and the increased secretion of adrenalin through which it is maintained. In conditions of the animal when no other responses of the brain stem are being conducted, the vasomotor response also fails to appear.

76 (1658)

Preliminary report on a typhoid bacteriophage.

By **ANNE KUTTNER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

I would like to report briefly on a lytic principle isolated by the d'Herelle technique from the stool of a typhoid convalescent, kindly sent to me by the Research Laboratory of the Health Department. A small particle of feces was emulsified in broth and incubated overnight. The next day about twice the volume of broth was added and the emulsion was centrifuged and filtered through a Berkfeld. The original filtrate was both inhibitory and lytic, that is, a small amount of the filtrate added to a tube of broth would, in spite of heavy inoculation with the homologous typhoid strain, prevent growth, and young turbid broth cultures became transparent on the addition of small quantities of the filtrate. The lytic principle could then be transmitted in series from both the inhibited and the dissolved cultures.

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[1] Cannon, W. B., 1915, "Bodily Changes in Pain, Hunger, Fear and Rage," pp. 38 and 40.

[2] Elliott, T. R., *Journ. Physiol.*, 1914, xvix, 38.

The lytic principle thus obtained corresponds, for the most part, to those described by d'Herelle. The action is non-specific. It acts on Shiga and Mt. Desert dysentery cultures, as well as on the homologous strain of typhoid and on other typhoid strains. It has no action on the strains of Para A and B that I have tried, and I have not undertaken any experiments to see whether the lytic principle could become acclimatized to these organisms as described by d'Herelle. It also has no action on *B. Coli communis*, or *communior*. It does not seem to dissolve or inhibit Gram positive organisms such as the pneumococcus.

It is fairly thermostable. It is not destroyed by a temperature of 70° C. for 30 minutes. It loses its activity, however, after an exposure at 75° C. for 30 minutes. The lytic principle does not maintain its activity for any length of time in sterile broth, and cannot be transmitted in series in this medium. It does not dissolve killed cultures, whether killed by heat or by the action of ether, and is not transmissible from killed cultures. The lytic principle, furthermore, does not persist and cannot be transmitted in Berkfeld filtrates of young typhoid cultures, which contain a certain amount of bacterial protein in solution. Actively growing young typhoid cultures are essential for the activity of the lytic principle.

The dissolved or inhibited cultures usually do not become absolutely sterile. If subcultures are made, it will be found that the control will give a typical confluent growth, whereas the tubes containing the lytic principle will give a small number of discrete colonies, usually occurring at the very margin of the slant. These discrete colonies are usually of two types, one the round typical typhoid colony, the other an extremely irregular jagged colony. If these two types are fished to broth, it will be found that the fishing from the round colony will cloud the broth, whereas the fishing of the irregular colony will often remain clear after 12 to 18 hours in the incubator. The lytic principle can be transmitted in series from the broth fishing of the irregular colony in the same way as from the original stool filtrate. Two types of colonies are also obtained from dissolved or inhibited Shiga and Mt. Desert cultures, one the bearer of the lytic principle, the other apparently a normal colony. The lytic action of these irregular colonies whether they be derived from a typhoid, Shiga or Mt. Desert cultures act in the same way. The lytic principle in the experiments thus far has showed no variations due to the fact that it was carried along by typhoid or dysentery cultures. I have, therefore, worked almost exclusively with the derivatives of dissolved typhoid cultures. The stock typhoid and dysentery cultures used in these experiments have been repeatedly streaked out without obtaining the irregular type of colony which is the bearer of the lytic principle. This type of lytic colony has, as far as I know, not been described by d'Herelle, but was first reported by Bordet in connection with the lytic principle that he was able to produce in the peritoneum of guinea pigs by repeated injections of *B. Coli.*

If one of these irregular colonies, whether typhoid or dysentery, is streaked out, a certain number of typical round colonies develop which, when fished into broth, will make the broth turbid. Typical colonies have never, in my experience, given anything but typical colonies on restreaking. Usually on restreaking an irregular "lytic" colony the majority of colonies obtained will be of the lytic variety, although I have found it very difficult to gauge the proportion of typical and lytic colonies that will be obtained from any given lytic colony. If a series of lytic colonies are obtained in this way in a row, it will be found, on examining under the microscope, that there are often minute transparent masses between the lytic colonies, and have been called "appearances" by previous observers. On examining the irregular lytic colonies under the microscope it will be found that the lytic colonies owe their irregular shape to the fact that their edges have faded out into these transparent "appearances." It is impossible to predict the amount of "appearances" that will be obtained by restreaking lytic colonies, and varying degrees of transparency occur in the "appearances." Fishings of the most transparent type of "appearances" have failed in most instances to produce any growth on a variety of media. When growth did occur it proved to be colonies of the lytic type. Comparative fishings of lytic colonies and "appearances" into young turbid broth cultures of typhoid were made to see if the lytic principle was carried by the "appearances"; the former usually became transparent after several hours incubation, whereas, the latter, except in rare instances, failed to clear up. The few cases where the addition of "appearances" to the turbid broth cultures seemed to exert a dissolving action can, in my opinion, best be interpreted as lytic colonies almost completely dissolved, but which still contained a small number of living bacilli carrying the lytic principle. All attempts to find a definite structure in the "appearances" by different methods of staining have failed. Plates have been observed at frequent intervals, to see if the amount of "appearance" increased, but this was never the case. Whether the "appearances" do not increase because the lytic principle dissolves up all the susceptible bacilli present as fast as they grow, and reduces them to " appearances," and has no action on the so-called resistant bacilli which form the bulk of the lytic colony, is still to be determined. On the other hand, it might be argued that the "appearances" do not spread on further incubation because by that time the typhoid bacilli have grown too old to be susceptible to the action of the lytic principle. From my observations to date, although very incomplete, I have found nothing to indicate that these "appearances" represent living structures, they suggest much more that the same phenomenon which is indicated by the clearing of the broth when the lytic principle is acting in fluid media occurs also on solid media.

It seems possible to me that when a fluid culture is rendered transparent it means that the susceptible bacilli present in the originally turbid emulsion are reduced to the state of "appearances." A chemical analysis of the end product of the dissolved culture compared with a similar analysis of the "appearances" if they can ever be obtained in sufficient quantity, ought to prove this point. I have experiments of this nature planned at the present time.

The potency of this lytic principle has not increased from one generation to another as indicated by d'Herelle in certain instances but has remained the same over a period of three months. The original stool filtrate, dissolved culture derivatives, derivatives of broth fishing of lytic colonies all appear to be about equally active. I have carried out experiments to see whether the potency of the lytic principle depended to any extent on the amount of protein dissolved, and have, therefore, compared the lytic action of an inhibited culture with a freshly dissolved culture. In the former case the amount of bacterial protein inoculated was very small compared to the amount of bacterial protein in the turbid culture. After 4 hours when the turbid culture had become transparent and the inhibited culture was clear, whereas, the control for the inhibition experiment already showed definite growth, both tubes, the dissolved and inhibited culture, were filtered and the lytic action of each determined in a series of dilutions. There was practically no difference in the activity of the two tubes in the interval that the tubes were observed. I intend to repeat this experiment, using a greater range of dilutions and observing at more frequent intervals. However, the amount of bacterial protein dissolved does not seem to influence the activity of the lytic principle very strikingly.

This lytic principle is very active in a dilution of 1–10, dissolving a turbid culture in from 2 to 4 hours. In a dilution of 1–100 the culture is usually dissolved in 12 hours. But in most cases the balance between the lytic action and the overgrowth by the resistant bacilli is temporary. Sooner or later, in most instances, the resistant bacilli win out, and make the transparent culture cloudy again. I have made fishings of the resistant types and tried the action of the lytic action on these bacilli. I have found that it often takes longer for the lytic principle to dissolve a resistant type than to dissolve the stock culture, but that, eventually, it seems to clear up a culture of this sort also. The resistant culture, on transplanting, seems to lose its resisting ability, but I have not finished working on this point.

I have tried to find a temperature where the lytic principle was still active and the bacilli could no longer multiply, so that, if the tubes had once become transparent, they would remain so. I have found that the lytic principle acts more quickly at a temperature of 41°–42°, a turbid culture will become transparent in half the time required for a similar tub at 37° C. These experiments are still under way, and it is very much of a question whether it will be possible to get the lytic principle to work where there are no actively growing bacilli. Between 45° and 50° the lytic principle is not active.

The most striking single fact about these lytic principles is that they are only active when added to young growing cultures. I obtain the best results in lysis experiments where I wash up the growth of a young agar culture in broth and then add enough of this heavy emulsion to 10 c.c. of sterile broth tube to make it definitely cloudy. A large amount of unused culture fluid media media favors the reaction enormously. I have tried adding drops of a heavy young typhoid emulsion to a freshly dissolved transparent culture until it is again turbid, but the lytic principle which can be demonstrated to be active on other turbid young broth cultures in a dilution of 1–100, will be unable to dissolve 0.2 or 0.3 c.c. of a young typhoid emulsion in 10 c.c. of lytic principle unless fresh nutritive material is added.

Another extremely important fact about this lytic principle (similar observations have been made by other workers), is that one single contact with the lytic principle is sufficient to divide a normal culture into two types, one the typical colony, the other atypical carrying the lytic property. This can be demonstrated both in fluid and in solid media. If the lytic principle is added in a dilution of 1–10 to a turbid culture, and the culture is shaken and plated immediately, the two types, in some instances, will be obtained. If an active filtrate is allowed to drop on a young agar growth of typhoid, the culture will be dissolved at this point, and, if the plate is incubated for another day, a few lytic colonies may develop in this area. The so-called resistant bacilli must be present in the original culture, together with the susceptible bacilli, since, in the case where the broth culture is plated immediately, the resistant bacilli have not had time to become hardened to the action of the lytic principle.

The above findings have simply been enumerated without any attempt to develop a theory. The data on the subject is still accumulating too rapidly for me to take definite sides.

77 (1659)

The effect of Heat and Age upon the Antiscorbutic Vitamine in Tomatoes.

By **MAURICE H. GIVENS** and **HARRY B. McCLUGAGE.**

[*From the Department of Physiology, University of Rochester, Rochester, N. Y., and the Research Laboratories, Western Pennsylvania Hospital, Pittsburgh, Pa.*]

Some time ago we proved[1] that the raw tomato is an efficient antiscorbutic agent; that the fruit can be subjected to a temperature of 55–60° C. for 14–24 hours or 35–40° C. for 36–44 hours and still retain a significant content of its antiscorbutic vitamine; and that such heat treated material is still potent after three month's ageing. Simultaneously Hess[2] proved that canned tomatoes are effective as antiscorbutic agents for children and guinea pigs; and later[3] he showed that tomatoes "canned almost a year previously were noted not to have their antiscorbutic value diminished appreciably by this ageing."

The value of the tomato as an antiscorbutic agent having been proved, it is highly desirable to have determined the effect of heat and age upon the antiscorbutic accessory in the fruit. With this end in view feeding experiments have been conducted on guinea pigs. In this way we have found guinea pigs protected against scurvy by daily doses of 2.5 grams of fresh raw tomatoes; by 10 grams of fresh raw tomatoes heated one hour at 100° C.; by 2 grams of dried tomatoes heated fifteen minutes at 100° C.; by 10 grams of tomatoes canned at fifteen pounds pressure for thirty minutes; by 3 c.c. of commercial canned tomatoes three years old; and by 10 grams commercial canned tomatoes, three years old, cooked fifteen minutes at 100° C.

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[1] Givens, M. H., and McClugage, H. B., PROC. SOC. EXP. BIOL. AND MED., 1918, xvi, 2.

[2] Hess, A. F., and Unger, L. J., PROC. SOC. EXP. BIOL. AND MED., 1918, xvi, 1.

[3] Hess, A. F., and Unger, L. J., *Am. J. Dis. Children*, 1919, xvii, 221.

78 (1660)

The effects of citrates, malates and phosphates upon the calcium balance and the calcium content of the blood.

By **GUY W. CLARK** (by invitation).

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley.*]

Most of the literature concerning the action of the citrates, malates, phosphates deals with the toxicity, tolerance, excretion and cumulative effects of these substances. The experiments briefly presented in this paper were carried out to ascertain whether the repeated administration of various acid radicals, commonly classified as "calcium precipitants," would, (1) result in a permanent decrease of the calcium content of the blood and, (2) if the calcium balance would be affected in any way.

Rabbits receiving a calcium-rich diet (daily intake of 0.44–1.24 gm. of Ca[1] were used as experimental animals. The calcium balance was determined at weekly intervals; the calcium content of the whole blood and plasma at intervals of 3–7 days.

CITRATES

Five rabbits received 5–45 daily (subcutaneous) injections of 0.26–1.23 gm. per kg. of one of the following: tri-sodium, tri-potassium, di-sodium citrate and citric acid. The experiments show that all of the animals maintained a highly positive balance and that there were no abnormal variations in the calcium content of the blood. On the first day of the injection period one animal received (during 1½ hours) two subcutaneous doses (50 c.c. each) of 4 per cent tri-sodium citrate. Thirty minutes after the second dose the plasma showed a decrease of 14 per cent. in its calcium content. Four days later the same animal received two doses (50 and 55 c.c.) of the same solution of sodium citrate. Thirty minutes after the first injection the plasma showed a decrease of 16 per cent. in its calcium content. Autopsy was performed on three of the animals and no macroscopic lesions of the urinary system were found. Polyuria was a usual result—definite diuresis was observed in only one case.

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[1] See under Phosphate for exceptions.

MALATES.

Two rabbits received 12–33 daily doses (subcutaneous) of 0.16–1.10 gm. per kg. of 4 per cent. di-sodium malate. Both animals maintained a highly positive calcium balance. No abnormal variations in the calcium content of the blood were observed. Autopsy of one animal showed no macroscopic lesions of the urinary system.

PHOSPHATES.

Four rabbits received 5–34 daily doses (subcutaneous) of 0.16–0.51 gm. per kg. of 4 per cent. di-sodium phosphate. All of these animals maintained a highly positive calcium balance even though two of them were on a much lower calcium intake. (Rabbit No. 5, 0.13–0.16 gm. Ca per day; Rabbit No. 10, 0.17–0.31 gm. Ca per day.) Five days after receiving the first injection of phosphate, Rabbit No. 5 showed a decrease in the calcium content; 35 per cent. in whole blood and 28 per cent. in the plasma. Six days after receiving the first phosphate injection, Rabbit No. 10 showed a marked decrease in the calcium content of blood (23.0 per cent.) and plasma (30 per cent.). The average of 8 determinations during the injection period (35 days) showed a decrease in the calcium content of 20 per cent. in whole blood and 26 per cent. in plasma.

An intermittent albuminuria was observed in all animals receiving phosphates.

SUMMARY.

1. Repeated subcutaneous injections of citrate, malate or phosphate have no effect on the calcium balance of animals receiving a calcium-rich diet.

2. The calcium content of the blood may be temporarily decreased by the injection of large doses of citrate.

3. Animals on a low calcium intake may, after phosphate injection, exhibit a decrease of 20–26 per cent. in the calcium content of the blood and still maintain a positive calcium balance. This observation emphasizes the suggestion that animals may adjust themselves to different "calcium levels" just as is true of nitrogen.

SCIENTIFIC PROCEEDINGS

Abstracts of Communications.

One hundred fourteenth meeting.

College of Physicians and Surgeons, New York City, March 16, 1921. President Wallace in the chair.

79 (1661)

Growth on diets containing more than ninety per cent. of protein.

By **THOMAS B. OSBORNE** and **LAFAYETTE B. MENDEL.**

[*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*]

Although it has been demonstrated that a carnivorous animal can be kept alive and maintained in activity for considerable periods on an exclusive diet of meat it is not known whether growth as well as maintenance can proceed on a regimen entirely free from both fats and carbohydrates. Hammarsten has stated that omnivora and herbivora cannot survive on such a ration. The few experiments on record in relation to this problem have without exception been conducted on a wrong plan, the food mixtures being inadequare in respect to one or more essential factors. Our successful experiences in growing rats on foods extremely poor in fats[1] and in carbohydrates[2] respectively encouraged us to test diets containing only minimal quantities of *both.* The mixtures included protein 95 per cent., inorganic salts 5 per cent., along with a supply of vitamins A and B in the form of tablets of alfalfa (0.4 gm.) and dried brewery yeast (0.2 gm.) daily. On such diets, when casein furnished the protein component, animals have already grown to three times their weight at the beginning of the trial. The vitamin-bearing substances were the only noteworthy sources of either fat or carbohydrate, and supplied 4–8 per cent. of the food eaten. Whether rats will attain adult size and normal function on such diets, furnishing protein as the almost exclusive source of energy and tissue substance, remains to be determined further. If future experiments prove as successful as those here described various problems of nutrition and physiological function can be approached from new experimental standpoints.

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[1] Osborne and Mendel, *J. Biol. Chem.*, 1920, xlv, 145.

[2] Osborne and Mendel, Proc. Soc. Exper. Biol. and Med., 1921, xviii, 136.

80 (1662)

The addition of yeast to a milk diet.

By **PHILIP B. HAWK, CLARENCE A. SMITH,** and **OLAF BERGEIM.**

[*From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia.*]

The experiments were made on white rats, one group of rats being fed a diet of pasteurized milk and a second group being fed a milk and yeast diet. The rats receiving the yeast made more satisfactory growth gains than did the rats receiving no yeast. Inasmuch as milk has been shown to be low in the water-soluble "B" vitamine, which is present in high concentration in yeast, it would seem that yeast may be found to be an important dietary adjunct for use in baby feeding.

81 (1663)

The rate of fixation of complement at various temperatures.

By **R. L. KAHN.**

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.*]

This investigation embraces three types of complement-fixing substances: (1) those elicited in rabbits due to injection of purified proteins; (2) those produced in the same animals due to injection of bacteria, and (3) those found in the serum of syphilitic patients. The antigens employed in the first two cases were specific, while in the case of the syphilitic sera, non-specific; four different Wassermann antigens being employed with each serum. Three fixation temperatures—water-bath, room and ice-box—were resorted to. Some phases of this investigation are still in progress and in this preliminary report, the work with the purified proteins only will be reported, although our findings indicate that the rate of fixation of complement is the same, no matter what type of fixing antibody is used.

Two purified proteins were employed: Edestin obtained from hempseed and phaseolin obtained from the kidney bean. These were kindly furnished by Dr. Thomas B. Osborne. Two rabbits were immunized with edestin and two with phaseolin. In order to elicit quantitative differences in the antibody production in the rabbits, four modes of immunization were resorted to. The edestin rabbits were injected intravenously according to "Immunization Methods No. 1 and No. 2," respectively, described by Kahn and McNeil in another paper.[1] The phaseolin rabbits were injected intraperitoneally. One rabbit received 100, 150, 200, 250 and 300 mgm. of phaseolin at 48-hour intervals, and the other 100, 150 and 200 mgm. of this protein at 24-hour intervals.

The complement fixation experiments were carried out in one tenth quantities of regular Wassermanns, otherwise in the usual manner, with 2 units of complement, 2 units of amboceptor and 0.1 c.c. of a standard 5 per cent. suspension of sheep-cells. The respective antigens were prepared by weighing out 10 mgm. of the protein and dissolving these in 10 c.c. of $N/1000$ NaOH to which was added 0.05 c.c. of $N/10$ NaOH. The alkali was necessary in order to get the proteins in solution. One c.c. of this protein solution was added to 9 c.c. of saline and 0.1 c.c. of this final solution (0.01 mgm. of the protein) was used in the tests. The serum dilutions employed in the tests were the following: 0.01 c.c., 0.007 c.c., 0.004 c.c., 0.003 c.c., 0.002 c.c., 0.001 c.c., 0.0005 c.c., 0.0003 c.c., and 0.0001 c.c.

After establishing the presence of specific complement fixing antibodies in the rabbit's sera by preliminary tests, fixation experiments were carried out with the serum dilutions indicated above, varying both the lengths of time and the temperatures of fixation.

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[1] *J. Immunol.*, 1918, iii, 281.

Thus the first series were run both at water-bath and ice-box temperatures with the following fixation periods: 15 minutes, 30, 45, 60, 90 and 120 minutes. In view of the fact that complement has a tendency to be destroyed when exposed for too lengthy periods in the water-bath, the fixation tests at this temperature were not extended beyond 2 hours. Neither were the fixation periods extended beyond this time when the fixations were at room temperature. In the case of the ice-box, however, the fixation periods were continued for 3, 4, 5 and 6 hours, and occasionally longer. At the end of each fixation period, standard amounts of sheep cells and amboceptor were added to each set and placed in the water-bath to determine whether or not the complement had been "fixed."

The results indicate:

(1) That the phenomenon of fixation of complement goes on equally well at water-bath, room or ice-box temperature.

(2) That from 50 to 75 per cent. of fixation takes place during the first hour and that fixation is completed in the neighborhood of 4 hours at ice-box temperature.

82 (1664)

The quantitative relation between complement and complement fixing antibody.

By **R. L. KAHN.**

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.*]

In the course of investigation on precipitin and complement fixing antibodies produced by injections of edestin, it was observed that, while the serum of a rabbit immunized with this protein showed the presence of precipitin antibodies, it did not show any complement fixing antibodies when employing the usual 2 units of complement in the fixation tests. It appeared reasonable at first to accept this finding as evidence of the lack of relation between these two types of antibodies. It seemed, however, that possibly the employment of 2 units of complement in the tests might give a sufficient excess of this ingredient to render a serum negative for complement fixing antibodies, although a reasonable number of such antibodies might still be present in the serum. With this possibility in mind, a series of complement fixation tests were carried out, using complement gradations of 1¼ units, 1½ units, 1¾ units and 2 units. The quantity of serum employed was 0.01 c.c.

It was observed that the same serum which gave negative results when 2 units of complement were employed, gave weak positive results with 1¾ units of complement; stronger positive results with 1½ units; and still stronger with 1¼ units of complement. In the last case the serum showed slight anticomplementary properties, which disappeared after about 10 minutes' incubation in the water bath.

This work is still being continued, but the results obtained thus far indicate that the employment of 2 units of complement in complement fixation tests is too great an excess of this ingredient for correct results in some cases, and that the employment of lesser quantities of complement, properly controlled, would serve as a finer measure of the complement fixing power of a given serum.

83 (1665)

The thermostability of complement fixing antibodies resulting from protein immunization.

By **R. L. KAHN.**

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.*]

In a series of studies on the rate of destruction of antisyphilitic complement fixing substances by heat, recently reported by Kahn and Boyd,[1] it was observed that in practically all cases these substances were destroyed when subjected to temperatures ranging from 60 to 65 degrees C. These results were obtained by heating a number of fractions of syphilitic sera at different temperatures in the water bath and running regular Wassermann tests with each fraction. It was felt, however, that the results obtained with the complement-fixing substances present in the sera of syphilitic patients could not be applied to specific complement fixing substances obtained after protein injections, in view of the fact that, in the former case, the antigens employed were non-specific. This has led us to study the rate of destruction by heat of *specific* complement-fixing antibodies.

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[1] Kahn and Boyd, *Proceedings Society of Amer. Bacter.*, Dec., 1920. Abstr. of Bact., forthcoming Issue.

The mode of immunization as well as the complement fixation tests were conducted as indicated in the first paper of this series. The tests were carried out in each case with unheated serum and the same immune serum heated to varying temperatures, beginning with 5 minutes at 56 degrees C. and ending with 1 hour at 65 degrees C. It was soon found, however, that these temperatures did not lessen the antibody content of the rabbit serum, and that the thermal destructive point of these complement fixing antibodies existed apparently at a higher temperature level.

The sera were then diluted 1–10 with saline in order to raise the protein coagulation level (Eberson) and placed in the water bath for 2 hours at 65 degrees C.; 1 hour at 70 degrees C.; and ½ hour at 75 degrees C.—without any apparent effect on the antibody content. The sera were then subjected to temperatures of 80 degrees C. and 85 degrees C. for 15 minutes. At the former temperature the antibodies were practically destroyed, while at the latter, completely destroyed.

The fact that the so-called complement fixing antibodies present in the sera of patients suffering from syphilis are destroyed when subjected to temperatures ranging between 60 and 65 degrees C. and that specific complement fixing antibodies withstand a temperature of 75 degrees C. suggests the possibility that there exists inherent biological differences between the two types of antibodies and opens a suggestive field for research.

84 (1666)

The use of blood plasma in the imbedding or the dissection of small organisms.

By GEORGE A. BAITSELL.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Various methods have been used in order to overcome the difficulties attendant upon the carrying of a minute organism, such as a protozoön, through the numerous reagents necessary for imbedding in paraffine preliminary to microtome sectioning. It has been found that the phenomenon of coagulation in blood plasma makes it of use in work of this type. In carrying out this method, a few drops of blood plasma is placed on a depression slide under a binocular microscope and the specimen at once placed in it with a pipet. After the plasma coagulates, which will take place in a very few minutes, the specimen will be found to be firmly imbedded in a resistant fibrin clot which can be taken through the various reagents, imbedded in paraffine, sectioned in any desired plane and finally mounted on a slide. In brief, the clot containing the specimen may be treated as a regular piece of tissue of the same size.

The orientation of the specimen in the clot may be accomplished before the clot is fully formed or at the time when the clot is in the clearing fluid just prior to imbedding. In the clearing fluid the clot becomes transparent and can be examined under a microscope and the imbedded specimen located in it. The clot can then be trimmed so as to indicate the orientation of the specimen.

Plasma of various animals can be used. I have generally used frog plasma and have secured it by the method previously described.[1]

Blood plasma has also been found to be useful in holding small animals firmly in a certain position so that they can be dissected. In this connection it may be noted that the dissection can proceed as far as desired and then no matter how fragile the dissected parts may be, additional plasma can be added and the fragile dissected part or parts imbedded in a clot in the same manner as described above. If desired, the clot can then be taken through the regular reagents and imbedded in paraffine for sectioning.

There is no doubt that blood plasma can be put to many other similar uses depending upon the nature of the problems and the ingenuity of the investigator. The securing and keeping of the plasma will not be found too difficult after the operation has been performed a few times.

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[1] *Journal of Experimental Medicine*, 1915, xxi, 456.

85 (1667)

Antipneumococcus protective substances in normal chicken serum.

By **CARROLL G. BULL** and **CLARA MCKEE**.

[*From the Department of Immunology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.*]

The serum of the domestic fowl has been found to protect mice and guinea pigs against infection with the pneumococcus. The protective power of the serum was demonstrated by giving mice, intraperitoneally, 1 c.c. of the serum and giving the infecting culture by the same route four to six hours later. The following protocol illustrates the plan of the experiment and the results obtained.

PROTOCOL.

MOUSE PROTECTION AGAINST TYPE I PNEUMOCOCCUS WITH NORMAL CHICKEN SERUM.

Mice.	Serum.	Type I Pneumococcus.	Results.
1.....	1 c.c.	0.01 c.c.	D. 48 hrs.
2.....	1 c.c.	0.001 c.c.	S. 5 days
3.....	1 c.c.	0.0001 c.c.	S. 5 days
4.....	1 c.c.	0.00001 c.c.	S. 5 days
5.....	1 c.c.	0.000001 c.c.	S. 5 days
6.....	—	0.0000001 c.c.	D. 36 hrs.

D., dead; S., survived 5 days and discarded.

Further experiments showed that the serum protects against all serologic types of pneumococci and that there is a particular protective substance for each type. Thus, serum adsorbed with Type I organisms no longer protects against this type but protects against Type II cultures as well as before the adsorption and vice versa. This relation exists between all of the types.

A peculiar relationship was found to exist between Type II strains and the subtypes of this group. Thus, serum adsorbed with Type II organisms failed to protect against Type II culture but still protected against the cultures of Types II *A* and II *B*. Serum adsorbed with Type II *A* organisms loses its protective power for II *A* and II cultures but not for II *B* cultures. Also, serum adsorbed with II *B* organisms no longer protects against

II *B* and II cultures but still protects against II *A* cultures. Hence, in terms of the protective substances in chicken serum, Types II *A* and II *B* are main groups with Type II as a subgroup to them both.

Fractionation of the serum by means of ammonium sulphate and dialysis showed that the protective substances adhere quantitatively to the water-insoluble globulin.

86 (1668)

A simple apparatus for studying the factors influencing fish migration.

By **F. E. CHIDESTER.**

[*From the West Virginia University, West Virginia.*]

At the request of Dr. H. M. Smith, commissioner of fisheries, certain studies have been made at the Woods Hole Laboratory of the Bureau of Fisheries on the factors influencing fish migration.

In testing reactions to various salts a simple apparatus was devised that seems to eliminate some of the drawbacks of the well-known Shelford apparatus.

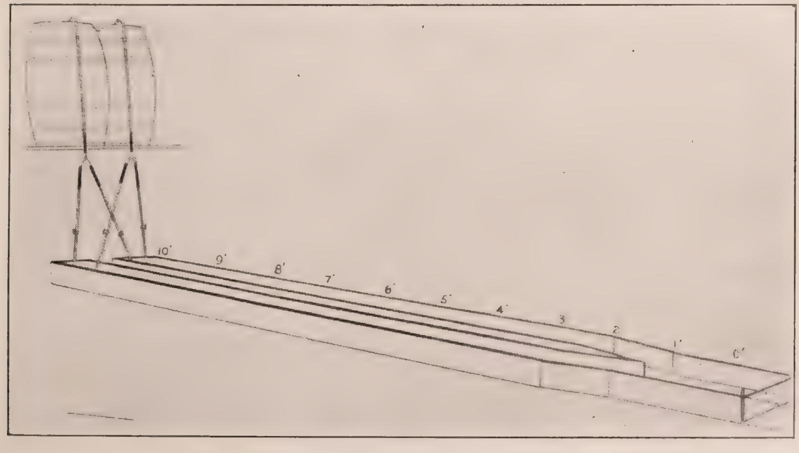

Fig. 1.

It consists (Fig. 1) of two troughs running almost parallel into a large trough which has a movable partition permitting the water to escape at its sides. By a simple arrangement of stopcocks it is possible to change the inflow of any substance from one trough to the other very quickly. It is also possible to secure conditions similar to those in the Shelford apparatus by using an intake at the end of the large trough, thus furnishing three intakes, two of them parallel to each other. In this case the movable partition is not used, the outflow being in the middle of the larger trough.

The apparatus used during the summers of 1919 and 1920 is 20 feet long. The dimensions of the receiving trough are 10 feet in length, 8 inches in depth and 8¾ inches in width. The two tributary troughs are each 10 feet in length, 4 inches in depth and 4½ inches in width. The tributary troughs have been elevated varying degrees so as to cause the water to flow downwards into the receiving pool. In addition to the use of barrels as containers for the solutions used, in some experiments the inflow has been directly from the circulation pipes of the laboratory.

The fish used in the experiments included *Fundulus heteroclitus*, *Fundulus majalis*, and *Clupea harengus*. They were tested with sea water of various dilutions and concentrations, *m*/10 solutions of NaCl, KCl, $MgCl_2$, $CaCl_2$, $MgSO_4$, various combinations of those salts and also with sewage polluted water. The temperature and the stream flow were varied in some of the experiments.

The results obtained indicate the following behavior:

1. The fish used in the experiments (10 fish for 25 trials) responded to both toxic and nontoxic substances, discriminating them readily under the same conditions of stream flow and temperature.

2. With a more attractive stream flow, usually one slightly more rapid than the control, the fish were lured into solutions that were quickly lethal in experimental jars. No evidence of intoxication in reactions to any particular substance was adduced. The reaction seemed to be one of pure rheotaxis.

3. Temperature change was readily detected but the optimum temperature varies with the season and the physiological condition of the animal.

4. In experiments with the apparatus used, errors due to the reaction of the fishes to movements of the observer have been

reduced by the arrangement of the observing post at the ends of two almost parallel troughs. The reaction of a fish to currents of water has also been considered in furnishing it control and experimental flows close together.

5. Habit formation has been studied by changing the control to the experimental trough after a series of trials with any of the substances.

87 (1669)

Pharmacological examination of cinnamein, benzyl succinate and benzyl nitrite.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University.*]

The discovery of the interesting pharmacological properties of benzyl benzoate and benzyl acetate first announced by the author in these Proceedings and the widespread therapeutic use of benzyl benzoate which immediately followed it, has naturally stimulated various observers to search for other benzyl compounds which might be available for clinical work. A number of such compounds have been since described, but none of these to the author's knowledge possessed together the two characteristics of benzyl benzoate, namely simplicity of chemical structure and efficiency of pharmacological action. The present author has also examined a number of benzyl preparations and found a majority of these unimportant in comparison with the original drug. In the present communication, however, it is proposed to describe briefly three benzyl preparations which have more than scientific interest in as much as at least two of these may be suitable for therapeutic application in special cases.

Benzyl benzoate is a synthetic compound, but is also found in nature and it has been suggested that for the production of the benzyl effect *Cinnamein* might be used. Cinnamein is a mixture of esters, alcohols and other substances obtained from the balsams of Peru and Tolu. Among these are benzyl benzoate and *benzyl cinnamate.* Cinnamein is a drug possessing a pleasant aromatic odor and bitterish taste and is anesthetic to the tongue and lips.

Pharmacological experiments with it showed distinct benzyl effects on smooth muscle organs. The toxicity of the preparation was, however, found to be greater than that of benzyl benzoate, for rats, guinea pigs and cats, the ratio of toxicity between cinnamein and benzyl benzoate being three to two. This drug was administered therapeutically to a number of cases. It produced very much the same effects as benzyl benzoate, but was found to be very much more irritant to the stomach and therefore its use was discontinued.

While benzyl benzoate is not disagreeable to the taste of most people, there are individuals who are nauseated by some of the preparations on the market. For the treatment of such cases attempts have been made to synthesize solid benzyl compounds. A number of these have been examined by the author and with one exception, *benzyl succinate*, were found to be inert. This is a beautiful crystalline powder, soluble in alcohol, ether and chloroform and practically insoluble in water. It melts at 49°–50° C. This drug is soluble also in olive oil. Experiments with benzyl succinate have shown that it produces typical benzyl effects on smooth muscle when ingested by mouth and when applied to isolated tissues, but to a much lesser extent. This compound seems to break up much more slowly than the benzoate when taken into the body and as a consequence its action is much milder than that of benzyl benzoate. The toxicity of this compound for rats and cats was found to be practically the same as that of benzyl benzoate. A number of clinical tests were made with the drug. It was found to be much less effective than benzyl benzoate in cases of spasmodic dysmenorrhea and still less so in cases of bronchial spasm and angiospasm. The best therapeutic results with this compound were obtained in gastro-intestinal cases. Here a milder action than that of benzyl benzoate was produced but the effects were possibly of longer duration owing to the slow breaking up of the drug.

One of the indications for the therapeutic use of benzyl benzoate originally described by the author was of angiospasm or vascular hypertension. While benzyl benzoate was found to be effective in many such cases, other patients failed to react to the drug. It was deemed desirable to combine the anti-spasmodic benzyl effect with the vasodilator action of the nitrites and the author decided to study the pharmacology of *benzyl nitrite* in this connection. Benzyl nitrite is a definite chemical compound described by a number of chemists. It is a yellow liquid, boiling at 115½° to 116° C. at 35 millimeters pressure. The drug is slightly soluble in water, but freely soluble in alcohol. While pure benzyl nitrite rapidly decomposes on exposure to the air, alcoholic solutions of the same keep fairly well. Laboratory experiments with benzyl nitrite revealed the typical benzyl effects on smooth muscle organs both in vitro and in vivo. The effect on blood pressure showed a rapid fall, but unlike the case of sodium nitrite and nitroglycerin the vaso dilatation was of much longer duration. The toxicity of benzyl nitrite is not very great, but on intravenous injection the nitrite effect on the blood is manifested after large doses (100 millimeters per kilo weight of dog or cat). An alcoholic solution of the drug has been administered to a number of patients by mouth for the relief of excessive hypertension. The results so far obtained have been very satisfactory but the investigation has not yet been completed and further work on the subject is in progress.

88 (1670)

New method for graphic study of heart murmurs.

By HORATIO B. WILLIAMS.

[*From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York.*]

Methods hitherto in use have proved unsatisfactory for recording murmurs, except such as are loud and low-pitched or of a sustained musical character and not very high-pitched. Einthoven's method, in which a carbon microphone is used, gives murmur records which are often more complicated than the sounds themselves, a circumstance which appears to be due to lack of damping in the moving parts of the microphone, especially of the carbon particles. In most microphones the diaphragms have a natural period too low for best results, but the undamped motion of the carbon particles is the most serious drawback. The methods not involving use of a microphone all suffer from a common defect. They are insensitive if the instruments are made of high natural period and if made sensitive they give records so distorted as to be almost valueless except for time-relation studies of the first and second sounds and of very intense murmurs.

To overcome these difficulties an electromagnetic telephone has been used to convert the sound oscillations into electrical oscillations. This telephone is provided with an air-damped diaphragm of high natural period. A shallow ring fastened to the telephone cap serves as "mouthpiece" and has a small lateral opening to maintain atmospheric pressure within it except for the sudden variations due to sound. This device is applied directly to the chest wall and the air column confined between the diaphragm and the chest wall is very short and has a high natural period.

The currents produced by the telephone are amplified by a four-stage amplifier used in such a manner as to give practically distortionless amplification. It is necessary to protect the vacuum tubes against extraneous sound and mechanical vibration. It is also necessary to shield all parts of the circuit against electrostatic disturbances and to keep the amplifiers away from sources of magnetic disturbance or else shield against them also.

The recording instrument is a string galvanometer. The preliminary work has been done with the natural period of the galvanometer approximately .002 second. This is too slow for best results and an instrument of higher period will be substituted in future work. Between the galvanometer and amplifier a condenser is placed in series, its capacity being so chosen relative to the other parts of the circuit that the comparatively low-pitched first and second sounds are partially suppressed in the electrical record while pulsations due to the higher pitched murmurs are transmitted undiminished. It is thus possible to produce the necessary amplification of the murmur record without the enormous excursions of the galvanometer which the first and second sounds would otherwise produce. A further advantage of this selective sound filter is that the appearance of the record corresponds more closely with the impression which the sounds make on the ear.

The general character of the first, second and third heart sounds as rendered by this method *without* the selective filter is in close correspondence with the curves recorded by the method of Einthoven. The murmurs are rendered better than by the microphone and the disturbances due to undamped motion of diaphragm and carbon particles being absent, the murmur records look less complicated.

A more detailed description with constants of the instruments and a discussion of results will be communicated after measurements with a galvanometer of higher natural period have been carried out.

89 (1671)

Concerning toxic byproducts of bacillus botulinus.

By **J. BRONFENBRENNER, M. J. SCHLESINGER** and **P. F. ORR.**

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School.*]

Attempting to shorten the incubation period of botulinus toxin by injecting gradually increasing amounts of toxin into mice, one of us (Orr) has observed early that when the amount of culture filtrate injected reach 0.5 c.c. animals frequently died within a few minutes after injection. Further study of the nature of this intoxication has brought out that when *B. botulinus* is grown on suitable medium there are produced, in addition to the specific toxin, other poisonous products. If such a culture is filtered through a Berkfeld candle and the filtrate treated with alcohol these secondary toxic products remain in solution. The alcoholic extract equivalent to 0.5 c.c. of the original filtrate is fatal when injected intraperitoneally into mice of 17–22 grams.

The first symptoms are noted immediately after injection and consist of restlessness and marked contraction of the abdominal muscles. Within a minute or two the animal shows increased response to external stimuli especially to sharp sound. Shortly the animal becomes prostrated. The increased excitability persists a few minutes longer, the respiration increases in depth and decreases in frequency, and may become as infrequent as 5 inspirations a minute. The animal goes into coma interrupted by sharp convulsive seizures with contraction of the extensor muscles throughout the body. Death occurs in 5 to 15 minutes after the injection and during one of these convulsive seizures. If less than a lethal dose is injected the animal may exhibit all the symptoms described above but will recover completely in 2 to 4 hours.

The toxic substances responsible for these acute symptoms are quite distinct from the specific botulinus toxin and are not neutralized by the specific antitoxin. Besides we obtained similar products from the cultures of "atoxic" strains of *B. botulinus*, as well as from those of *B. sporogenes*, *B. tetani* and *B. proteus* when these organisms were grown on medium composed of minced meat broth.[1] Moreover, these toxic products are not of the nature of bacterial toxins since they are dialyzable, they act only in very large amounts (0.5 c.c. as compared with 0.002 c.c. of botulinus toxin) and since they exert their action immediately upon injection and not after a period of incubation characteristic of all bacterial toxins. In addition the toxic substances in question are thermostable and are not destroyed even in the autoclave when heated in a sealed tube. In the open container, however, and especially in presence of strong alkali, their toxicity diminishes with coincident volatilization of basic products. From the above it is evident that the toxic substances in question are chemical byproducts of the bacterial metabolism.

We feel justified in reporting these observation for several reasons: In the first place, the presence of toxic substances in cultures of different proteolytic bacteria have been at different times mistaken for specific toxin.[2] Secondly, in connection with the analysis of partly decomposed food products in which the presence of toxins is suspected, one usually injects into a test animal a comparatively large amount of the food extract. Since such extracts may contain poisonous salts, we wish to emphasize the necessity of controlling such animal inoculation by antitoxin neutralization experiments. When the latter is not available the test of thermostability of the toxic substances in neutral as well as in alkaline reaction may indicate the true nature of the substance.

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[1] When grown on beef infusion broth or peptone water without minced meat, neither of these organisms produce the toxic substances in question.

[2] Barger, G., and Dale, H. H., *Brit. Med. Journ.*, 1915, 11, 808.

90 (1672)

The food reactions of the infusorian Spathidium spathula.

By LORANDE LOSS WOODRUFF and HOPE SPENCER.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Spathidium spathula is an holotrichous infusorian whose sole diet consists of smaller ciliates, chiefly species of *Colpidium*. If the truncated anterior end of a *Spathidium*, which is swimming forward and revolving on its long axis, happens to come into direct contact with a *Colpidium*, the latter usually becomes motionless at once and its protoplasm shows signs of pathological changes.

Paralysis and death of the prey is apparently brought about by the liberation of a specific substance from the oral region of *Spathidium* which is toxic to small ciliates. Various authors have ascribed this result to trichocysts about the peristome, but as a matter of fact none are present. However, a number of rod-like bodies can be demonstrated in the thickened rim of the peristome which may represent the seat of the poison. One is at liberty to interpret these bodies as "trichocyst material," though the poisonous nature of trichocysts, even in *Paramecium*, remains to be demonstrated.

The paralysis of a *Colpidium* by a *Spathidium* results in a marked change in the latter's behavior, which up to this instant has comprised random swimming movements. If the *Colpidium* happens to remain against the truncated anterior end, the *Spathidium* forthwith proceeds by means of the thickened edges of the peristome to force its prey down through the slit-like mouth into the interior of the cell. On the other hand, if the *Colpidium* is not instantly rendered motionless and becomes removed a short distance from the oral region of its captor, the latter institutes a series of rapidly repeated avoiding reactions. This behavior has a tendency to keep the animal in the general vicinity of its quarry because each time the Spathidium starts away its forward progress is checked by the reaction. It is obvious that sooner or later, merely on the basis of chance, the anterior end of the animal will again come in contact with the *Colpidium*, but a careful study of many captures shows beyond doubt that the apparently random reactions are successively modified so that the peristome of *Spathidium* is brought gradually nearer and nearer until it grasps the *Colpidium.* In brief, *Spathidium* in the majority of cases recovers its lost prey, even though the latter may have become removed a distance equal to several times the length of the *Spathidium.*

The factors involved here may be products derived from the dead *Colpidium*, but more probably are directly related to the poisonous secretion liberated by *Spathidium* itself. In any event, the complex series of reactions exhibited by *Spathidium* afford, it is believed, a remarkable example of sensing at a distance by a unicellular organism.

The complete paper will appear in the Journal of Experimental Zoology.

91 (1673)

The rôle of the nervous system in the regulation against cold.[1]

By **H. G. BARBOUR** and **E. TOLSTOI.**

[*From the Department of Pharmacology, Yale University School of Medicine.*]

One of us has shown that a dog placed in an ice-water bath up to the neck exhibits as one of the reactions to cold a concentration of the blood, losing approximately 10 per cent. of its blood fluid. Such extreme temperatures, however, even well-nourished dogs are unable to resist, the rectal temperature falling rapidly.

Investigating further the mechanism of regulation against cold we attempted to find first a bath temperature which, while not sufficiently cold to lower the body temperature, would arouse distinctly the regulation against cooling. Secondly, on dogs deprived of their nervous regulatory mechanism by section of the cord between the sixth and seventh cervical segment, it was attempted to determine whether the failure to resist the temperature of baths not too cold to be normally withstood is associated with inability to concentrate the blood.

It has been found that normal dogs will exhibit a fairly efficient regulation against cooling in a bath the temperature of which is maintained for a half hour or more at 20° C. In two such dogs there were net gains in body temperature as a result of exposure to the bath (respectively 0.1° C. and 0.4° C. in forty minutes). In a third animal the temperature fell 1.5° C. during the first twenty minutes but in the following thirty-five minutes showed a gain of 0.8° C., thus showing that the regulation against cooling was established after a delay.

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[1] From investigations aided by the Elizabeth Thompson Science Fund.

In these three normal experiments the increases in blood solids were 1, 2.1 and 1.8 per cent. respectively. The following table illustrates these points. In all of the experiments here reported the room was kept at a standard temperature of 22° C.

TABLE I.

EFFECTS OF 20° C. BATHS IN NORMAL DOGS.

Dog Number.	Percentage Blood Solids.		Rectal Temperature, ° C.			
	Before.	After.	Before.	After 20 Min.	After 40 Min.	After 55 Min.
48........	19.3	20.3	39.9	40.1	40.0	....
23........	20.4	22.6	38.3	38.4	38.7	....
27........	14.7	16.5	39.4	37.9	38.4	38.7

With the above should be contrasted the effects of similar baths upon three dogs with transection of the cervical cord, as shown in Table II.

TABLE II.

EFFECTS OF 20° C. BATHS UPON DOGS WITH CERVICAL CORD SECTION.

Dog Number.	Days After Operation.	Percentage Blood Solids.		Rectal Temperature, ° C.	
		Before.	After.	Before.	After 40 Min.
21[1]........	1	19.5	19.9	34.5	31.0
42.........	7	18.1	18.9	36.5	32.6
27.........	2	17.3	17.6	37.5	34.6

The second table shows that dogs with cord section between the sixth and seventh cervical segment are quite unable to withstand baths of 20° C., exhibiting a rapid fall in body temperature, usually to a dangerous level within forty minutes. Unlike the normal dogs they fail to exhibit shivering. As regards the concentration of the blood, all of these dogs exhibited an abnormally low blood solid percentage as a result of the operation.

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[1] Bath 17.5° C.

The cold stimulus was unable to evoke more than a very weak response. This inability to reduce the blood volume and thereby diminish the flow through the body surface helps to account for the very rapid loss of body temperature. In the second cord dog the regulation appeared somewhat better; this experiment was, however, performed seven days after operation instead of one or two days, as in the other experiments, and there may have been time for some readjustment of the development of the mechanism. This increase in blood solids from 18.1 to 18.9 per cent. in forty minutes did not, however, suffice to prevent the rapid fall in body temperature.

Dogs made poikilothermic by cervical cord section are deprived of reactions which may be set up between the temperature sense nerve endings and the circulation. The shifting of water from the blood to the tissues is evidently such a reaction. It is therefore concluded that the rôle of the nervous system in the reaction against cold is to convey impulses from the temperature sense nerve endings to "heat centers" which in turn, besides shivering and vasoconstriction, incite blood thickening. Hemo-concentration lessens the water available either for heat dissipation by evaporation or for providing blood bulk enough to flood the peripheral vessels.

92 (1674)

The effects of environmental temperature changes upon blood concentration.[1]

By H. G. BARBOUR.

[*From the Department of Pharmacology, Yale University School of Medicine.*]

Experiments upon normal dogs, kept at rest in hot and cold baths up to the neck, have shown regular changes in total blood solids. Blood solids were determined simply by weighing a sample of 15–16 drops shed freely from the ear vein and drying to constant weight.

Exposure to hot baths for various intervals is illustrated by Table I, to cold baths by Table II.

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[1] From investigations aided by the Elizabeth Thompson Science Fund.

TABLE I.

HOT BATHS.

Dog Number	3	3	3	3	4	4	6
Initial bath temp.	40° C.	41.5°C.	41° C.	40° C.	40° C.	40° C.	40° C.
Percentage of Blood Solids.							
Before bath	21.6	21.5	21.4	22.2	19.3	17.5	19.4
After 10 mins. in bath				21.6	18.7		
" 15 " " "	21.2	21.1	20.3			16.5	
" 25 " " "				20.6			
" 30 " " "	21.6[1]		19.5				
" 40 " " "					16.9		
" 1 hr. 45 mins. in bath							18.8
Out of bath (and cooled with blower,) 25–45 min.	22.2		22.2				

TABLE II.

COLD BATHS.

Dog Number	3	3	4	8[2]	9[2]	9[2]
Initial bath temp.	11° C.	8° C.	10° C.	...	...	...
Percentage of Blood Solids.						
Before bath	20.5	19.6	17.3	20.3	20.5	20.4
After 10 mins. in bath		21.7				
" 15 " " "	22.4		18.8			
" 25 " " "		21.7		22.3		
" 30 " " "						21.0
" 35 " " "	22.5					
" 45 " " "					21.5	
Out of bath, 20–40 mins.	20.8	21.2	19.3			

It will be seen from the tables that normal dogs respond regularly to a moderately high environmental temperature by hemodilution and to a cold environment by hemo-concentration. Roughly, the change usually approximates 2 per cent. of the total blood weight, which means a 10 per cent. change in the fluid content of the blood.

The circulatory factor in regulation against overheating and cooling consists not merely in transferring of blood respectively to or from the body surface but also in actual shifting of water into or out of the blood stream. The response to a hot environment is peripheral vasodilation *plus* hemodilution; a cold environment evokes vasoconstriction *plus* hemoconcentration.

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[1] Bath had cooled down to 37.5°.

[2] Under morphine and chloretone. Cold stimulus was ice-water sponge and blower.

To simplify the above presentation the rectal temperature readings have been omitted; these showed in the case of the cold baths and sometimes in the case of the hot, that the bath conditions were too extreme for the animal to withstand in spite of the regulatory responses.

Confirmatory evidence has been accumulated since the above experiments were carried out.

93 (1675)

The life history of an amicronucleate race of Didinium nasutum.

By **MARY W. PATTEN.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

A series of pedigree lines of *Didinium nasutum*, consisting of the progeny of a single exconjugant, were bred continuously under practically constant environmental conditions from December 16, 1919, to September 10, 1920.

A cytological investigation of the preparations made daily from stock left after each isolation, demonstrated the absence of a morphological micronucleus in vegetative, dividing and conjugating animals. In the race from which these pedigree lines were derived by conjugation, and also in other unrelated races of *Didinium*, micronuclei were easily demonstrable. It is clear, therefore, that the parent race was micronucleate, while one of its progeny, a single exconjugant (the original cell of the race under investigation) was amicronucleate. Moreover, this amicronucleate condition persisted throughout the life of the race, that is, through 652 generations.

At various intervals during the life history of this culture, periods showing a tendency for encystment and conjugation occurred, but the animals which encysted or conjugated invariably died, a fact undoubtedly related to their amicronucleate condition. Rhythmical periods of depression followed by increased vitality, which resembled those shown by Woodruff and Erdmann[1] to be associated with endomixis in *Paramecium caudatum* were also observed. Endomixis, however, if such occurs in the free living state of *Didinium*, could not be carried out in this race during the rhythmical periods owing to the lack of a definitive micronucleus.

The amicronucleate state without doubt arose from some irregularity during conjugation. Prandtl[2] in his study of the cytology of conjugation in *Didinium* found individuals in which all the micronuclei had become transformed into macronuclei, but he thought animals so endowed were incapable of living. The present study shows that they are viable but unable to undergo processes such as encystment, conjugation, and endomixis which are dependent largely upon micronuclear activity.

94 (1676)

Calcium in the blood.

By **WM. C. THRO** and **MARIE EHN.**

[*From Cornell Medical College, New York City.*]

One year ago we read a paper before this society on the "Calcium in the Blood of Patients with Furunculosis and Acne." We were aware that we obtained very large amounts of calcium in the blood of some of the patients, but were unable to detect our error. At that time we used the gravimetric method and with it were unable to detect any calcium in the filter paper used, although we made six attempts. This year, after perfecting ourselves in the titration method for calcium determination, as given by Halversan and Bergeim,[3] we found that some of the filter paper we had been using contained considerable amounts of calcium. We then decided to abandon the gravimetric method and have used the titration method in obtaining the results given here.

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[1] Woodruff, L. L., and Erdmann, R., "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exp. Zoöl.*, 1914, xvii, 425–518.

[2] Prandtl, H., "Die Konjugation von *Didinium nasutum*," *Arch. f. Protistenkunde*, 1906, vii, 229–258.

[3] Halversan, J. O., and Bergeim, O., *Jour. Biol. Chem.*, 1917, xxxii, 159–170.

We have made nearly one hundred determinations of the amount of calcium in human blood in the following conditions:

Acne	23	Nephritis	3
Diabetes	40	Normal	7
Furunculosis	9	Pneumonia	3
Miscellaneous	8	Tuberculosis (cured)	1

The figures given are milligrams per 100 c.c. of blood plasma:

Acne 12.1, 10.0, 9.0, 4.5, 6.8, 8.3, 11.3, 10.6, 10.6, 9.8, 8.3, 9.8, 6.0, 4.9, 11.0, 9.1, 13.2 (16.6, 16.5 repeated), 13.2, 15.9, 12.1, 7.1.

Furunculosis 6.9 (Dr. J. 1.1, February 11; 5.9, March 11), 9.4, 9.1, 12.0, 8.0, 8.1, 9.8.

Normal 9.8, 9.4, 7.9, 9.1, 9.3, 8.3, 7.8.

Diabetes (8.7, January 27; 9.4, January 31; 7.9, February 9; 1.1, February 11), 5.0, 10.0, (10.0, January 31; 3.4, February 28), 4.8 ,(7.5, February 15; 10.6, February 25; 11.1, March 2), 9.8, 8.7, (8.8, February 4; 10.2, February 16), 9.8, (7.2, February 8; 6.0, February 11; 9.1, March 2), 8.7, (11.0, February 9; 12.8, February 14), 12.5, 10.2, 9.8, 9.1, 11.0, (9.8, February 21; 10.6, February 25), (7.9, February 25; 5.3, March 7), 3.7, 10.0, 8.0, 8.0, 5.9, 11.1, 5.3, 16.7; 11.1.

Hemophilia 5.1; purpura hemorrhagia 7.4; hemiplegia 9.8, 9.8; secondary anemia 10.2, 10.2 repeated; nephritis 11.7; 11.1, 8.4; pulmonary tuberculosis, healed, 14.3; pneumonia 7.8, 9.1, 8.4; familial paralysis 8.6, 9.0 repeated.

The calcium content of the blood of patients with diabetes varied considerably, but with most of them the amounts were normal or below normal and very few were above normal. If such patients were put on a restricted diet the amount of calcium in the blood usually became lower.

Our chief aim was the determination of the calcium content of the blood of patients with acne and furunculosis. We found that the calcium content of the blood was high in patients with very severe acne who had not been treated, but there were great variations in the amounts of calcium in the blood of acne patients. Four patients with furunculosis had reduced amounts of calcium in the blood. One patient had a very slight amount, *i.e.*, 1.1 mg. in 100 c.c. of plasma, and one month later, after calcium sulphide therapy, the amount had increased to 5.9 mg. per 100 c.c.

The figures in the braces represent determinations on the same patient on different dates.

95 (1677)

Studies in the regeneration of blood.

By **E. M. K. GEILING** and **H. H. GREEN.**

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

In continuation of the unpublished studies of Jencks in this laboratory upon blood regeneration in the rat, it is desired to report the following data: The normal erythrocyte count of rat's blood ranges between 7.5 and 10.5 millions per cubic millimeter; and the hemoglobin content between 110 and 140, as determined by the Smith-Cohen method. Data from rats of varying ages and different sex appear to fall within these limits.

Single hemorrhages, equivalent to 2 per cent. of the body weight, and double hemorrhages, of 2 per cent. on two successive days, were carried out. With the latter procedure it was found possible to reduce the erythrocyte count and hemoglobin content to about one third normal.

On the usual mixed food regeneration of blood was complete in from 7 to 10 days after single hemorrhage, and in 10 to 14 days after double hemorrhage; erythrocyte count and hemoglobin content being taken as indices of regeneration. During starvation regeneration followed single hemorrhage in normal time, but was accompanied by heavy loss in body weight. In starvation following double hemorrhage, the animals usually succumbed before regeneration was complete. Splenectomized rats appear to regenerate in normal time on the normal diet. Rats reared upon the Osborne-Mendel fat-deficient diet showed normal erythrocyte count and hemoglobin content, and after double hemorrhage regenerated in normal time upon the same diet. On a diet deficient in either protein, vitamine, or mineral matter, blood regeneration was appreciably delayed, after double hemorrhage. Hence the data at present available suggest that although blood is parasitic upon the other tissues, any one of the three dietary factors just mentioned can be made a limiting factor in the rate of regeneration, if the hemorrhage is sufficiently severe. Experiments are now in progress to determine the rôle of iron in blood regeneration.

96 (1678)

Studies on the lytic agent of Bordet and Ciuca.

By **ANDRÉ GRATIA.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

We owe to the kindness of Doctor Bordet a strain of *B. coli* with which he carried on his studies, a certain quantity of the corresponding lytic agent and a typical mucoid strain of his modified *coli*. With this material we have observed the following facts.

1. The inhibition produced by the lytic principle on the growth of *B. coli* is greatly influenced by the reaction of the medium: faint in a slightly acid (P_H 6.8) or neutral (P_H 7.0) or even slightly alkaline broth (P_H 7.4), it is much stronger in a more alkaline medium (P_H 8.0 or 8.5).

2. We have isolated from the original strain of *B. coli* two types of organisms: the one, Type S,[1] is sensitive to the lytic agent; the other, Type R,[1] is much more resistant. These types are distinguished also by other characteristics: type S grows quickly in artificial medium and is non-motile; type R grows more slowly, is extremely motile, much less phagocytable and more virulent. Both types ferment carbohydrates, saccharose excepted; type R decolorizes neutral red, type S does not. Both types keep their individuality even after passage through a guinea pig.

3. The original lytic agent was found to be specific; it acted exclusively on the coli with which the guinea pigs had been injected. By allowing this original lytic principle to act on broth cultures of our two types of *B. coli*, we have obtained two new filtrates. The first, resulting from dissolution of the sensitive

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[1] S = sensitive; R = resistant.

strain S, is specific as is the original filtrate. But with the second, obtained from the resistant strain R, Doctor Wollstein of the Rockefeller Institute has found a marked action on Shiga, on Flexner and on Hiss dysentery bacilli. In consequence of this observation, we have been able, by a method of successive passages through appropriate strains, to extend the lytic power to other species, as typhoid and paratyphoid bacilli, and have obtained by this somewhat different technique results similar to those recently published by Bordet and Ciuca.[1]

4. We have observed also that the modified *coli* of Bordet and Ciuca, *e.g.*, the *coli* which has resisted the lytic action, contains two types of organisms: a mucoid and fluorescent type, *coli* M 1, and a non-mucoid and translucent type, *coli* M 2. Both types, once isolated, keep their individuality after many passages in artificial media, but if the non-mucoid *coli* M 2 is submitted again to the lytic agent, we find amongst the organisms which resist a certain number of mucoid bacilli.

97 (1679)

A dietetic production of rickets in rats and its prevention by an inorganic salt.

By **H. C. SHERMAN** and **A. M. PAPPENHEIMER.**

[*From the Departments of Chemistry and Pathology, Columbia University, New York.*]

The occurrence of rickets in white rats maintained under laboratory conditions has been well known to pathologists since the first publication of Morpurgo in 1900; and the essential identity of the lesions with those of human rickets has been established by the work of Morpurgo himself, of Schmorl, of Weichselbaum, and especially by the detailed histological studies of Erdheim. One of us (A. M. P.) also has had opportunity to become familiar with the disease in rats, in the course of an investigation of the possible influence of the thymus upon the teeth and skeletal system. In none of these investigations, however, were the dietary conditions of the rats standardized and controlled.

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[1] *C. R. Soc. belge Biol.*, T. 1921, lxxxiv, 278.

In continuing by means of feeding experiments upon rats the study of the mineral elements in nutrition which has engaged much of the attention of one of us for the past fifteen years, we have found a relatively simple diet which has led to the development of rickets in every one of the cases thus far examined; while complete protection was afforded by the addition of 0.4 per cent. of potassium phosphate (K_2HPO_4) to this diet, or (more strictly) by the introduction of this mount of potassium phosphate in place of a part of the calcium lactate which the rickets-producing diet contained.

In our experience healthy rats of families on normal diet when separated from their mothers at 28 to 30 days of age average about 40 grams in weight and a calcium content of about 0.3 gram or 0.7 per cent. If then placed upon good diet the calcium content of the body increases in greater ratio than the body weight so that the percentage of calcium in the body rises continuously until at about four to six months of age the adult percentage of calcium—about 1 to 1.25 per cent. of the body weight—is reached, after which the weight of the body and the weight of calcium which it contains continues parallel until growth is complete.

If, however, the healthy young rat after weaning is placed at the age of 28 to 30 days upon a diet consisting of patent flour and sodium chloride, growth in body weight is practically suspended and simultaneously the body practically ceases to increase its calcium content. In such case the rat usually lives about six weeks upon the experimental diet with hardly any change either in body weight or total body calcium. Such rats showed multiple fractures, marked deformity of the thorax, and osteoporosis.

In parallel experiments in which calcium lactate, with or without ferric citrate, was added to the diet of patent flour and sodium chloride (Diets 83 and 84), there was little or no growth in body weight but the body gained sufficient calcium so that its percentage of calcium after 4 to 6 weeks on this diet was about the same as in a normal rat of the same age, though the absolute amount of calcium contained in the body was much below that of a normal rat of the same age, as would be expected in view of the suspension of growth. These rats uniformly showed rickets.

When with all other conditions the same, the diet was modified by the introduction of 0.4 per cent. of potassium phosphate, (Diet 85) rickets was prevented. Here again growth in body weight was practically suspended, but the addition of potassium phosphate to the diet resulted in an increased assimilation of calcium so that together with absence of bone lesions there was found a percentage of calcium in the body somewhat higher than the normal average for the age, though naturally in view of the marked stunting of the animals the absolute amount of calcium in the body was less than in a normal rate of the same age (which would have had at least twice the body weight).

Our pathological findings are summarized in Table I.

TABLE I.

SUMMARY OF FINDINGS AS REGARDS RICKETS.

Diet No.	Composition of Diet.	Total Number of Rats Examined.	
		A. Rachitic.	*B*. Non-rachitic.
83	Patent flour....................95% Calcium lactate.................. 3% Sodium chloride.................. 2%	2	0
84	Patent flour....................95% Calcium lactate.................. 2.9% Sodium chloride.................. 2.0% Ferric citrate.................... 0.1%	13	0
85	Patent flour....................95% Calcium lactate.................. 2.5% Sodium chloride.................. 2.0% *Potassium phosphate*.............. 0.4% Ferric citrate.................... 0.1%	0	15[1]

In our observations as summarized in Table I, the diagnosis of rickets has been based upon the microscopic examination of ribs and femora, partially decalcified in Mueller's fluid; and in several instances, confirmed by X-ray examination. The histological criteria are: (1) the great increase in width of the zone of growing cartilage, and its irregular projection towards the diaphysis; (2) the failure of calcium deposition in the zone of preparatory calcification; (3) a pronounced increase in the osteoid margin, both in the region of the metaphysis, and along the shafts of the bones.

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[1] 5 of these placed on special diet at age of 60 days; 1 at 81 days; all remaining animals started at four weeks of age.

It is seen from the Table that rachitic lesions developed in 15 rats maintained on Diets No. 83 or No. 84 for a period varying from one to two months after weaning. The rats surviving the longer period, naturally, showed more extreme changes, but the lesions were easily recognizable in the X-ray and in the gross changes at the chondro-costal junctions, after four weeks. Nine rats maintained for similar periods on Diet No. 85, which differs from Diet 84 only in the substitution of 0.4 per cent. K_2HPO_4 for an equivalent amount of calcium lactate, showed entirely normal bones, both grossly and microscopically; and six other rats, five of which were changed to Diet 85 at the age of 60 days and one at 81 days, also failed to develop rickets.

While our work was in progress there have appeared the first two of a series of papers by McCollum and his associates dealing with the experimental production of rickets in rats by means of a wide variety of deficient diets. In the first of these papers McCollum and his co-workers state (p. 340) that they "are not willing to hazard any statements in regard to the factors operating to produce rickets in the child or the experimental animal"; while in the second paper, published simultaneously, the same authors say (p. 344) that by the use of faulty diets "especially certain diets deficient in the socalled fat-soluble A or in both that substance and calcium, the cartilage and adjacent portions of the metaphysis of the long bones of the extremities could be rendered entirely free from calcium deposits and a condition identical with the rickets of human beings be obtained."

In view of the emphasis thus given by these investigators, as well as by others, to deficiency of calcium or of fat-soluble vitamine or both as causing rickets, it has seemed to us that we might aid in the attack upon this puzzling disease by placing on record at this time our observations upon the prevention of rickets when caused by the particular experimental diet here described, by the simple introduction of a single inorganic salt, and that not a salt of calcium, into the diet.

Without entering into detailed discussion of these results at this time we desire to emphasize the following facts in order to avoid misunderstanding. On the particular diet here used rickets uniformly appeared in the absence, and was uniformly prevented by the presence, of the added potassium phosphate; but this does not imply that in these cases the cause of rickets was necessarily a deficiency either of potassium or of phosphorus. The quantitative relations of the inorganic ions rather than an absolute deficiency of any one of them, may have been the determining factor. Also it may well be that under certain dietary conditions, rickets may be caused by deficiencies or unbalanced quantitative relationships of organic as well as inorganic food factors. It would appear however to have been demonstrated that rickets may be caused or prevented without change in either the protein or vitamin components of the diet and therefore that neither of these can be regarded as a necessarily predominating factor. It is also of outstanding interest that the rats showing multiple fractures and marked deformity of thorax, which would probably be inincluded under the classification used by some writers as "presenting the gross picture of rickets" but whose bone lesions on microscopic examination were classified as those of osteoporosis, were those which had been subjected to even greater dietary deficiency than those showing typical rickets.

We are indebted to Dr. J. M. Steiner for his coöperation in the X-ray examinations and to Miss F. L. MacLeod for the quantitative determinations of calcium.

98 (1680)

Growth accessory substances in the nutrition of bacteria.

By **TH. THJOTTA** and **O. T. AVERY**.

[*From the Hospital of the Rockefeller Institute, N. Y. City.*]

In studying the growth characteristics of mucoid bacilli Thjotta observed that *B. influenzæ* will grow in blood-free broth containing the mucoid material from cultures of Friedlander's bacillus and other closely allied organisms. The growth accessory substance or substances which can replace blood and blood derivatives in the cultivation of Pfeiffer's bacillus Thjotta found in both the saline suspensions and watery extracts of the heat killed bacillary material. Furthermore, these bacterial emulsions and extracts can be boiled for ten minutes and filtered through Berkefeld candles without losing their growth-inducing properties. It was thought not unlikely that the growth-stimulating effect of these bacterial extracts might be due to substances belonging to the class of the so-called vitamines. To test this assumption, similar extracts were prepared from yeast cells which are known to be rich in growth accessory substances. These extracts, even in minute amounts, were found capable of promoting growth. Extraction of the growth accessory substances from another source, namely, green vegetables, was tried; extracts of fresh tomatoes, green peas and string beans were found remarkably active in stimulating growth. These active yeast or vegetable extracts when added to broth greatly accelerate growth of organisms such as *Bacillus influenzæ* and pneumococci, so that within five hours abundant growth is evident. In the case of pneumococcus, a seeding too minute to initiate growth in plain broth alone will amply suffice to induce abundant growth in the same medium if a small amount of extract containing these growth accessory substances is added. In the case of *Bacillus influenzæ*, when seeded from blood media, luxuriant growth occurs in plain broth containing yeast extract, while no growth takes place in the same broth without the addition of extract. The presence of these growth accessory substances in extracts diluted 1 : 1,000 suffices to stimulate growth under these conditions. However, for reasons to be discussed later, continued cultivation fails in broth containing only yeast or vegetable extracts.

While the nature of these growth accessory substances is not known, they are presumably analogous to the so-called vitamines. It has been found that they resist boiling for at least ten minutes, that they are destroyed by autoclaving, that they are extractable from fresh vegetables and from growing bacterial and yeast cells, that they are water soluble, that they pass a Berkefeld filter, and that extracts of these substances contain but little nitrogen—about 0.116 per cent.

In the application of this principle to bacterial nutrition, particular attention has been given thus far to the nutritional requirements of *Bacillus influenzæ*, since this organism belongs to a peculiar group of bacteria which heretofore have been considered obligate hemophiles.

Although *Bacillus influenzæ* will grow luxuriantly when transplanted from blood medium to plain broth containing yeast extract, cultivation cannot be continued more than one or two transfers in yeast broth alone. This suggested that possibly some other substance may be carried over from the original blood culture in an amount sufficient to supplement the yeast broth and that growth fails in succeeding cultures because this substance is either exhausted by growth or lost by dilution on subsequent transfers. For purposes of discussion this substance may be referred to as the X factor and the vitamine-like substance in the extracts as the V factor. Neither of these two factors by itself can sustain growth of *Bacillus influenzæ*. Evidently both are essential to growth and both occur in blood which is always used in the cultivation of *Bacillus influenzæ*. As previously pointed out, the V factor it destroyed by autoclaving. If, therefore, blood medium is autoclaved, it should no longer be able to support growth of *Bacillus influenzæ*. This is actually the case. If, however, the X factor has not been destroyed by heating then this same medium should be reactivated by the addition of fresh yeast extract. This also is the case. The growth accessory substance (the V factor) which is destroyed by autoclaving blood, can be supplied from other sources, such as yeast; and this substance is capable of reactivating a medium in which, as a result of heating, the X factor alone remains. Search is being made for the X substance in material other than body tissue. That crystalline hemoglobin itself, however, does not contain both of these essential substances, is shown by the fact that pure crystalline hemoglobin when added to broth fails to support growth of *Bacillus influenzæ* unless yeast or its equivalent in V substance is also present. This fact indicates that crystalline hemoglobin contains some of the X factor. Further studies are planned to determine the importance of this principle in the cultivation of other species of bacteria. From analogy with animal nutrition, it seems not unreasonable to suppose that nutritional deficiency in the cultivation of bacteria may be overcome by the addition to culture media of the appropriate growth accessory substances.

99 (1681)

A clinical method for the quantitative estimation of calcium in blood.

By **MAX KAHN** and **L. G. HADJOPOULOS.**

[*From the Department of Laboratories, Beth Israel Hospital, New York City.*]

To 1 c.c. of blood serum in a 10 c.c. test-tube, add 4 c.c. of a 1 per cent. solution of ammonium oxalate. Let stand for ½ to 1 hour, and centrifuge for 5 minutes. Pour off the supernatant fluid. Wash the precipitate three times with distilled water, recovering the precipitate by means of centrifugalization. Add to sediment 1 c.c. distilled water and transfer to a vitreosil crucible. The test-tube is washed with distilled water and the washings also collected in the crucible. The water is slowly evaporated, and the precipitate burnt in a strong flame until CaO is formed. Dissolve the ash in ½ c.c. N/50 HCl, add 1 c.c. distilled water and titrate excess of HCl with N/100 NaOH, using phenolphthalein as an indicator. The amount of CaO can now be calculated.

Eight analyses may be made by this method in one hour. Remarkably uniform results are obtained upon repeated examinations of the same blood. For clinical purposes, this method is of great assistance.

100 (1682)

The penetration of normal mucous membranes of the rabbit by Treponema pallidum.

By **WADE H. BROWN** and **LOUISE PEARCE.**

[*From the Rockefeller Institute, New York City.*]

The fact may be recorded that highly virulent strains of *Treponema pallidum* are capable of penetrating some portion of the genital mucosa of normal rabbits and setting up an infection without necessarily producing the first gross lesion at the portal of entry. This fact was recently determined in 9 rabbits and with two highly virulent strains of *Treponema pallidum*.

The experimental method employed was as follows: The sheath of the animal was drawn forward to form a pouch into which was instilled 0.05 c.c. of a testicular emulsion rich in spirochetes. About 30 seconds were allowed for the emulsion to spread before releasing the sheath. Most of the fluid then ran out and between 0.04 and 0.05 c.c. could be recovered showing that only a thin film of the emulsion was retained. Infection was first indicated by enlargement and induration of the inguinal lymphnodes and later by the development of a general lymphadenitis with syphilitic lesions in other parts of the body.

All animals thus far inoculated by this method have become infected. In some of them, enlargement and induration of the inguinal nodes was clearly recognizable within 24 hours after the application of the emulsion. Thus far (5 weeks) only one of the animals has developed a visible lesion on either the penis or the sheath, although several of them have characteristic lesions in the testicles and scrotum.

The observations on these animals are not yet complete and the full significance of the experiments cannot be ascertained until the course of the infection has been followed much longer. Similar experiments with other mucous membranes are in progress.

101 (1683)

Neoplasia in experimental syphilis.

By **WADE H. BROWN** and **LOUISE PEARCE.**

[*From the Rockefeller Institute, New York City.*]

Neoplasia as a sequel to syphilitic infection is not uncommon in man but has never been recorded in an experimental animal. Recently, we have observed an atypical growth arising from the scar of an old syphilitic lesion in the scrotum of a rabbit which may prove to be a neoplasm.

The animal was inoculated in the scrotum, June 16, 1916. Small chancres developed and then underwent spontaneous regression. Several months later, there was a recurrence and the lesion in the left scrotum persisted for some time. In October, 1920, there was a slight diffuse infiltration of the left scrotum and a small nodule appeared at the site of the old chancre. Although it was known that the animal still harbored spirochetes,[1] none could be demonstrated by dark-field examination of material from the nodule and it was excised for histological examination. There was a prompt recurrence and with the growth of the second cutaneous lesion, the left inguinal glands became markedly enlarged and indurated. Again no spirochetes could be demonstrated and the lesion with one of the adjacent glands was excised under ether anesthesia. Histological examination of the cutaneous nodules and gland showed a growth which presented more the appearance of a neoplasm than of a syphilitic lesion. It was composed for the most part of atypical epithelioid cells undergoing active proliferation and exhibiting marked invasive tendencies.

Meantime there was a second recurrence and extension of the skin lesion over the mid line at the pubis with enlargement and induration of the right inguinal nodes. A deterioration in the physical condition of the animal was then apparent and progressed very rapidly, culminating in emaciation, weakness, severe anemia, loss of sphincter control with some spasticity of the hind legs and the formation of trophic ulcers about the anus and sheath. On this account, the animal was etherized.

Post-mortem examination revealed a widespread distribution of nodules identical in character with the original lesions. These were most abundant in the liver and bone marrow but were also present in other organs such as the spleen, the lungs and the kidneys.

Transplantation of material from an inguinal node gave a vigorous growth in the original animal and attempts to transfer the growth to other animals have apparently been successful.

102 (1684)

The pharmacology of acetone.

By **WILLIAM SALANT** and **NATHANIEL KLEITMAN.**

[*From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

The action of acetone was studied on cats and dogs under light ether anesthesia as well as on the isolated heart of the frog and turtle. In the experiments on intact animals acetone injected intravenously produced a fall of blood-pressure amounting in some cases to fifty per cent., especially in dogs when given in a concentration of fifty per cent. in saline solution, and about 2–3 c.c. per kilo. But in a number of experiments smaller quantities of acetone injected in a concentration of 25 per cent. in saline solution produced a similar effect on the circulation. This occurred almost uniformly in cats. Recovery was prompt, as a rule, even when 50 per cent. acetone was given, but occasionally low blood-pressure persisted for 2–3 minutes when larger amounts were injected. The fall of blood-pressure was due to cardiac depression, as was indicated by the fact that there was a corresponding diminution in the volume of the kidney shown by oncometric studies. That acetone in certain concentrations depresses heart action was also observed in our experiments on the isolated heart. Ten per cent. acetone in Ringer solution produced in all our experiments arrest of the heart, and very frequently the same effect was obtained with a five per cent. solution of acetone. Recovery, however, was observed in all experiments, even when the heart was perfused with ten per cent. acetone for several minutes. Not infrequently a stimulating after-effect was observed. That no permanent injury occurred was further shown by the fact that repeated perfusion of the same heart was followed by recovery. With low concentration of acetone no change in the heart action was observed.

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[1] See account of rabbit No. 1, *Amer. Jour. Syph.*, 1921, v, 1.

Observations were also made on the behavior of the vagus mechanism under the influence of acetone. In dogs, after repeated injections of acetone, stimulation of the peripheral end of the cut vagus with the interrupted current of moderate strength produced prolonged inhibition of the heart. The vagus center seemed likewise to be affected by acetone as it was observed in some experiments that heart action became much slower, especially after large amounts were injected. But when acetone was introduced after previous double vagotomy retardation of heart action was hardly discernible.

The effect of acetone on respiration was much more pronounced than on circulation. In cats prolonged periods of apnea were observed after moderate amounts of acetone were injected intravenously. Apnea was usually preceded by decreased frequency and depth of respiration. In dogs the effects were not constant as in several cases moderate amounts of acetone produced stimulation of respiration at first. As larger quantities, however, were introduced the depressing effect became very marked. A frequent phenomenon in our experiments was the occurrence of Cheyne-Stokes respiration after repeated acetone injections. The greater depressing effect on respiration than on circulation was also shown when large amounts of acetone were injected. Blood-pressure remained moderately high for some time after respiration stopped.

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103 (1685)

The reaction of taurin with α-naphthylisocyanate.

By **CARL L. A. SCHMIDT.**

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley.*]

Although taurin differs from that particular group of amino acids which constitute the building stones of the proteins in that it contains a sulphonic instead of a carboxyl group, it nevertheless closely resembles the amino acids in its chemical properties. Like the amino acids taurin can be quantitatively estimated when in pure solution either by treating it with HNO_2 and determining the nitrogen set free or by titration with alkali in the presence of formalin. It possesses amphoteric properties[1] and reacts with many of the amino acid reagents. Thus Paal and Zitelmann[2] found that taurin reacts with phenylisocyanate to give α-phenylureidoethylsulphonic acid, Gabriel[3] and his associates prepared benzoyl- and phthalyl-taurin, and Bergell[1] has attempted to use β-naphthalinsulphotaurin as a means of estimating taurin in urine.

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[1] Winkelblech, K., *Z. physik. Chem.*, 1901, xxxvi, 546.

[2] Paal, C., and Zitelmann, G., *Ber. d. d. chem. Gesell.*, 1903, xxxvi, 3337.

[3] Gabriel, S., and Heymann, P., *Ber. d. d. chem. Gesell.*, 1890, xxiii, 157. Gabriel, S., and Colman, J., *Ber. d. d. chem. Gesell*, 1911, xliv, 3628.

We have found that taurin like the carboxy amino acids[2] reacts with α-naphthylisocyanate to yield the corresponding hydantoic acid. The barium salt of α-naphthylureidoethyl sulphonic acid was prepared by adding to a concentrated solution of taurin which contained an equivalent amount of KOH, 1¼ equivalents of α-naphthylisocyanate and the mixture was shaken at frequent intervals for an hour. The insoluble dinaphthyl urea was filtered off and on acidifying with HCl the solution thickened but the free hydantoic acid did not separate. On addition of $BaCl_2$ solution the barium salt of the hydantoic acid was precipitated. This was washed with small quantities of ice water and then with alcohol and was dried to constant weight. Estimations of C, H, and Ba gave (per gram of substance) the following results.

Found		Calculated for $Ba(C_{10}H_7NHCONHCH_2CH_2SO_3)_2$
C	0.419	0.431
H	0.039	0.036
Ba	0.187	0.189

104 (1686)

A cheap and convenient source for glutamic acid.

By **CARL L. A. SCHMIDT** and **G. L. FOSTER.**

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley.*]

One of the pressing needs of biochemistry and allied sciences is an adequate supply of pure amino acids. While methods for the isolation and purification of these substances are well known, nevertheless the labor and expense involved are in many instances prohibitive except for the production of relatively small quantities. With the exception of glutamic acid no use for amino acids other than for scientific purposes has been found and manufacturers have as yet been unable to apply the benefits of quantity production sufficiently to make these substances cheap enough for extensive investigations.

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[1] Bergell, P., *Z. physiol. Chem.*, 1916, xcvii, p. 260.

[2] Neuberg, C. and Manasse, A., *Ber. d. d. chem. Gesell.*, 1905, xxxviii, 2359.
Loewy, A., *Deut. med. Woch.*, 1905, xxxi, 1918.
Neuberg, C., and Strauss, H., *Berlin klin. Woch.*, 1906, xliii, 258.
Hirschstein, L., *Berlin klin. Woch.*, 1906, xlviii, 589.
Neuberg, C., and Manasse, A., *Berlin klin. Woch.*, 1906, xliii, 734.
Wohlgemuth, J., and Neuberg, C., *Med. Klinik*, 1906, ii, 227.
Neuberg, C., and Rosenberg, E., *Biochem. Z.*, 1907, v, 456.
Levene, P. A., and Van Slyke, D. D., *Biochem. Z.*, 1908, xiii, 440.
Hirschberg, E., Dissert. Berlin, 1910.

It was shown some years ago by Ikeda[1] that the taste of the constituents of the seaweed *Laminaria japonica* is due to the content of glutamic acid. This is to a certain extent also true of meat extract and allied preparations. The demand for condiments of this type has led to the production of the mono-sodium salt of glutamic acid on a scale large enough to make it relatively cheap.

Our experiments with Ajinomoto (a commercial preparation of sodium glutamate sold by S. Suzuki & Co., Tokio, Japan) were carried out with a view of using this substance as a cheap as well as a convenient source for pure glutamic acid. Our results show that this preparation consists largely of the mono-sodium salt of glutamic acid. To isolate glutamic acid, Ajinomoto is dissolved in a small quantity of water (600 c.c. for each 100 grams) and HCl is added in an amount equivalent to the amino nitrogen present. Purified charocal is then added, the mixture brought to the boiling point, filtered hot and the filtrate placed in the ice chest to crystallize. The crystals are drained, washed several times with small quantities of ice water until free from chlorides and dried. From the mother liquor and washings, after concentration, a second crop of glutamic acid may be isolated. From 100 grams of Ajinomoto 55 grams of glutamic acid were obtained. On the basis of the amino nitrogen content (6.55 per cent.) of Ajinomoto the yield corresponds to about 80 per cent. of the glutamic acid present. On account of the NaCl resulting from the liberation of the glutamic acid from its salt and other impurities which are present, further crystallization is not profitable. The purified product yielded the theoretical nitrogen value and a 5 per cent. solution in 10 per cent. HCl gave a specific rotation of $+31.5°$.

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[1] Ikeda, K., Communication to the 8th International Congress of Applied Chemistry, 1912, xviii, 147.

SCIENTIFIC PROCEEDINGS

One hundred fifteenth meeting.

ABSTRACTS OF COMMUNICATIONS.

University and Bellevue Hospital Medical College, April 20, 1921.
President Wallace in the chair.

105 (1687)

Tissue weight and water content in a tetracotyledonous mutant of Phaseolus vulgaris.

By J. ARTHUR HARRIS.

[*From the Station for Experimental Evolution,*
Cold Spring Harbor, L. I.]

In 1915 studies of the tissues of normal and variant bean seedlings were begun in an effort to explain the differential mortality with respect to morphological characters demonstrated in an earlier experiment.[1]

In a first paper[2] it was demonstrated that teratological seedlings show a lower capacity for the development of primordial leaf tissue than do normal individuals of the same strain grown under conditions as nearly as possible identical. In these first experiments the conclusions were based upon the green weight of primordial leaves.

In a second study, tissue weight determinations were based on the trifoliate leaves of the third node as well as on the primordial leaves of the second node.[3] In these two investigations we attempted to determine to what extent morphologically aberrant seedlings differ from the normal seedlings of the race to which they belong in their physiological characters in so far as these can be measured by the capacity for the production of tissue. The results indicated that teratological seedlings show a lower capacity for tissue production as measured both by green weight and dry weight in primordial and first compound leaves than do their normal controls.

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[1] Harris, J. Arthur, *Science*, 1912, N. S., xxxvi, 713–715.

[2] Harris, J. Arthur, *Genetics*, 1916, i, 185–196.

[3] Harris, J. Arthur, Brooklyn Botanic Garden Memoirs, 1918, i, 167–174.

In a subsequent series of investigations we have instituted comparisons between the highly abnormal seedlings of a tetracotyledonous race of Phaseolus[1] and the normal seedlings of the parental race from which it originated.

The tetracotyledonous race is characterized by a modal number of four cotyledons and four primordial leaves but both of these characters are highly variable.

Classifying the tetracotyledonous plants according to number of primordial leaves, we have the mean green weight and the mean dry weight of primordial leaf tissue in teratological and normal seedlings shown in the accompanying table.

The data are given as average weights per plant and per leaf. The average per cent. of dry substance is shown in the final column of the table. All the values are averages of constants based on samples of approximately 100 plants.

The data show that without exception the mean green weight and the mean dry weight per plant of primordial leaf tissue is lower in the tetracotyledonous race than in the normal race.

The mean percentage differences (obtained by using the constants for the normal plants as a base) for green weight per plant range from -3.10 for the plants with six primordial leaves[2] to -31.55 per cent. for group of plants with 2 primordial leaves.

The percentage differences for dry weight of primordial leaves in tetracotyledonous and dicotyledonous races vary from -7.93 per cent. for the group of plants with 6 primordial leaves to -32.55 for plants of the tetracotyledonous race with 2 primordial leaves.

It will be noted that the difference between the abnormal and the normal plants decreases as the number of primordial leaves on the abnormal plants increases.

The results for the average green and dry weight per leaf in the mutant and normal series fully substantiate the conclusions concerning the physiological differentiation of the two races to be drawn from the average weights per plant.

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[1] Harris, J. Arthur, *Proceedings National Academy of Science*, 1916, ii, 317–318. Harris, J. Arthur, Memoir, N. Y. Botanical Garden, 1916, vi, 229–244.

[2] Theoretically plants with 7 leaves should have shown a smaller difference than seedlings with six leaves but the number of seedlings available was not so large and the constant is therefore not as trustworthy.

The differences in the average percentage of dry substance vary considerably but it is impossible to state in the absence of probable errors that the ratio differs from class to class.

The foregoing results for a heritable abnormal race substantiate the conclusions concerning the association of physiological with morphological differences already drawn from a comparison of variant and normal individuals within the same race.

Number of Leaves of Abnormal Race.	Pairs of Plants.	Value per Plant.		Value per Leaf.		Per cent. Dry Substance.
		Mean Green Weight.	Mean Dry Weight.	Mean Green Weight.	Mean Dry Weight.	
2 Leaves						
Abnormal	196	.52390	.03790	.26195	.01900	7.3090
Control	196	.76285	.05645	.38145	.02825	7.4350
Difference		−.23895	−.01855	−.11950	−.00925	−.1260
Percentage difference		−31.55	−32.55	−31.55	−32.40	
3 Leaves						
Abnormal	500	.61086	.04174	.20360	.01392	6.8516
Control	500	.77094	.05606	.38550	.02806	7.2842
Difference		−.16008	−.01432	−.18190	−.01414	−.4326
Percentage difference		−20.74	−25.54	−47.16	−50.38	
4 Leaves						
Abnormal	700	.68947	.04587	.17238	.01147	6.6918
Control	700	.78631	.05501	.39318	.02751	7.0305
Difference		−.09684	−.00914	−.22080	−.01604	−.3387
Percentage difference		−11.97	−16.34	−55.95	−58.12	
5 Leaves						
Abnormal	500	.70542	.04740	.14108	.00948	6.7618
Control	500	.77152	.05504	.38580	.02754	7.1692
Difference		−.06610	−.00764	−.24472	−.01806	−.4074
Percentage difference		−8.60	−13.78	−63.46	−65.52	
6 Leaves						
Abnormal	300	.74746	.05146	.12456	.00860	6.9076
Control	300	.77206	.05596	.38603	.02796	7.2616
Difference		−.02460	−.00446	−.26146	−.01936	−.3540
Percentage difference		−3.10	−7.93	−67.66	−69.16	
7 Leaves						
Abnormal	138	.73120	.05570	.10450	.00800	7.6200
Control	138	.77850	.06140	.38930	.03070	7.8920
Difference		−.04730	−.00570	−.28480	−.02270	−.2720
Percentage difference		−6.00	−16.76	−73.10	−73.90	

106 (1688)

Preliminary note on chemical changes in the blood of syphilitics under arsphenamin treatment.[1]

By **CHARLES WEISS** and **ANNA CORSON**.

[*From the Dermatological Research Institute, Philadelphia.*]

Among the very few recent publications on the subject of the chemistry of the blood in syphilis before or during arsphenamin therapy, the first is that of Rappleye.[2] He determined the urea of the blood and the phenol-sulphon-phthalein elimination in the urine of a series of twenty paretic cases before, and at intervals of one half, three and twenty-four hours after intravenous administration of *diarsenol*[3] (0.5 to 0.6 gm.), and also of a series of ten patients who had been under treatment for a long time. He observed fairly normal values (8 to 20 mg. of urea nitrogen per 100 c.c. of blood) in all cases both *before* and *after* treatment.

Elliott and Todd[4] made similar studies before and after a course of six weekly intravenous injections of 0.5 gm. of arsphenamin in a series of twenty syphilitic young men without evident renal disturbance. They found the average urea content of the blood to be 30.7 mg. before and 34.4 mg. per 100 c.c. of blood after treatment. One case showed an increase of 19 mg. In another series of nine cases to whom injections were given *twice* a week with the same total dosage, the average blood urea was 33.7 mg. before and 35.3 mg. after treatment. Five of the nine cases showed increases of from 4 to 5 mg. of urea nitrogen per 100 c.c. of blood.

It is important to note that although these authors had selected cases which clinically or by the albumin test showed no signs of renal disturbance, their values for blood urea nitrogen *before injection* are more or less pathological and range from 27 to 43 mg. with an average value of 33.7 mg. per 100 c.c. of blood.

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[1] Investigation aided by funds accruing from the preparation of arsphenamin.

[2] Rappleye, W. C. Notes on the Effect of Intravenous Diarsenol. *Jour. Lab. and Clin. Med.*, 1919, iv.

[3] This is the Canadian brand of arsphenamin (salvarsan).

[4] Elliott, J. A. and Todd, L. C. Effects of Arsphenamin on Renal Function in Syphilitic Patients. *Archives Der. and Syph.*, 1920, ii, 699–702.

Bailey and MacKay[1] in a study of 25 cases of syphilis that had developed toxic jaundice during arsphenamin treatment, found values for blood sugar fairly normal, cholesterol very high whenever there were any signs of liver disturbance, and in the greatest majority of cases, values for urea nitrogen ranging from 22 to 49 mg. per 100 c.c. of blood, twelve out of the twenty-five showing figures above 30 mg. They observed similar increases in the uric acid and creatinin of the blood. It is important to note that although none of these cases showed any proteinurea before or during treatment, they were distinctly nephritic, as proven by these pathological figures and by the fact that they became debilitated when placed on a high protein diet.

The importance of studying the kidney function of syphilitics before and during arsphenamin treatment has not been recognized by syphilographers in general. Wechselmann,[2] early in the history of arsphenamin therapy, emphasized the importance of kidney insufficiency in syphilitics, and ascribed most of the fatalities to this defect. Kolmer and Lucké,[3] in a recent histopathological study, showed that even small (therapeutic) doses of arsphenamin and neo-arsphenamin when injected repeatedly into the veins of rabbits produced vascular and tubular changes in the kidneys, characterized as "nephrosis." "Focal areas cf cellular degenerations and necroses were frequently well marked, particularly in the heart and liver."

Scope and Method of Investigation.—The patients studied were two cases of tertiary syphilis with optic atrophy. They were kept in a ward on a hospital diet, low in proteins and fats and fairly uniform from day to day. Their water intake was also controlled. The blood specimens were always obtained three hours after a special, constant breakfast. The analytical methods used were those of Folin and Wu.[4]

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[1] Bailey, C. V. and MacKay, A. Toxic Jaundice in Patients under Antisyphilitic Treatment. *Archiv. Int. Med.*, 1920, xxv, 628–647.

[2] Wechselmann, W. Uber die Pathogenese der Salvarsantodesfalle. Berlin, 1913. Urban and Schwarzenberg.

[3] Kolmer, J. A., and Lucké, B. Summary of Experimental Studies on the Histopathologic Changes Produced by Arsphenamin and Neo-arsphenamin. *Archiv, Der. and Syph.*, 1920, ii, 289–291; *ibid.*, 1921, iii, 483–580.

[4] Folin, O. and Wu, H. A System of Blood Analysis. *Jour. Biol. Chem.*, 1919, xxxviii, 81–109; and *ibid.*, 1920, xli, 367–375.

After a preliminary period of observation of about one week, 0.6 gm. of arsphenamin (using ampules of the same lot number and produced at the Dermatological Research Institute) was injected intravenously in 120 c.c. of distilled water by means of a gravity apparatus. Details of the analyses are given in Table I.

TABLE I.

SHOWING CHEMICAL CHANGES IN THE BLOOD OF SYPHILITICS DURING ARSPHENAMIN TREATMENT.

Patient I: T. M., Male, Age 38—Optic Atrophy.

Date.	Total Non-Protein Nitrogen.	Urea Nitrogen.	Sugar.	Remarks.
10–25–20	24.2	11.1	85.1	
10–26–20	30.0	13.2	100.0	[2]
10–26–20	32.9	13.2	123.8	V[3]
10–27–20	36.8	20.6	130.2	V
10–29–20	31.4	17.2	133.8	
11– 2–20	39.5	14.3	115.6	
11– 4–20	33.4	14.9	103.9	
11– 8–20	34.3	22.4	131.6	
11– 9–20	23.2	8.1	133.3	[2]
11– 9–20	28.4	9.4	129.9	D[3]
11–10–20	44.3	14.8	121.2	
11–13–20	34.5	11.3	137.9	
11–16–20	32.6	12.6	137.9	
11–18–20	31.0	12.4	139.9	
11–22–20	32.4	18.1	121.6	
11–23–20	34.8	16.9	116.3	V[2]
11–24–20	38.2	18.3	117.6	
11–26–20	30.3	13.5	108.1	

Patient II: M. J., Male, Age 28—Optic Atrophy.

1–19–21	33.5	11.4	93.5	
1–20–21	32.1	11.4	88.3	
1–21–21	32.6	11.7	111.4	
1–25–21	30.7	12.2	75.5	[2]
1–25–21	32.8	15.5	153.8	V[3]
1–27–21	31.5	11.3	113.0	
1–28–21	31.5	12.6	89.9	
1–31–21	30.7	14.2	137.9	
2– 2–21	38.8	18.6	96.4	G
2– 4–21	35.8	17.5	119.0	
2– 7–21	30.9	14.3	107.5	

[1] All figures are given in milligrams per 100 c.c. of blood.

[2] Injection immediately *after* sample was drawn.

[3] Patient received an intravenous injection of 0.6 g. arsphenamin 3 hours *before* this sample of blood was drawn.

V = "reaction"—patient vomited.

D = severe reaction with vomiting, diarrhea and pain in the legs.

G = gastric crisis. C = slight reaction—chills.

Summary of Results.—During the period of observation preceding the injections the values for urea nitrogen, sugar, and total non-protein nitrogen of the blood were within the upper normal limits given by Myers.[1] Three hours after the intravenous injection of 6 decigram doses of arsphenamin, a slight rise in the total non-protein nitrogen of the blood (2 or 3 mg.) was observed in each of the two cases studied. This rise became more pronounced one week later, reaching a value of about 38.0 mg. per 100 c.c. of blood, and then gradually declined. The injections were repeated two weeks later. A more pronounced rise (up to 44 mg. per 100 c.c. of blood) was observed twenty-four hours later—the immediate increase (three hours after injection) being similar to that seen after the first injections. The changes in the blood-urea nitrogen were, in general, parallel to the curves of the total non-protein nitrogen. The individual values of the former occasionally reached as high as 23 mg. per 100 c.c. of blood and often constituted more than 60 per cent. of the latter. Marked increases in the blood sugar were seen three hours or within three to seven days after the injections. In one case the blood sugar was more than doubled three hours after the injection. In no case, however, did the blood sugar reach much above the upper normal limit,—the value in the last case referred to being 153 mg. per 100 c.c. of blood.

The authors wish to thank Dr. Jay F. Schamberg, Dr. John A. Kolmer, and Dr. George W. Raiziss of this Institute for their kind coöperation throughout the work.

107 (1689)

Chemical stimulation of the annelid nerve cord.

By **A. R. MOORE.**

[*From the Physiological Laboratory of Rutgers College, New Brunswick, N. J.*]

According to Maxwell's[2] classification, based on work with mammals, there are two classes of excitants for nervous tissue, viz: (1) those which act only upon the medullated fibers, such as the

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[1] Myers, V. C. Chemical Changes in the Blood in Disease. *Jour. Lab. and Clinic. Med.*, 1920, v, 344.

[2] Maxwell, S. S., *Am. J. Physiol.*, 1918, xlvii, 283.

calcium precipitants, (2) those which act on the gray matter only, as creatin and strychnin. In order to determine possible similarities and differences between mammalian nervous tissue and that of one of the annelids, experiments were carried out on the nerve cord of the earthworm, *Lumbricus terrestris*. In these experiments the worm was decapitated, the anterior end of the preparation pinned down and the nerve cord laid bare. The cord was then dissected free for a distance of about 20 segments and the stimulating substances applied directly to it. Stimulation was indicated by squirming movements of the posterior segments.

Excitants of the first class, KCl, $BaCl_2$, and Na_3 citrate, each in $M/8$ concentration, gave marked excitation. Of the excitants of the second class, camphor and strychnin, each in saturated solution, and picrotoxin crystals, all yielded positive results within a minute after application, but phenol, nicotin and creatin had no effect, used either as crystals or in solution. $M/64$ tetra-ethylammonium chloride gave strong stimulation.

The fact that excitants of the first class act on the annelid nerve cord shows that the nerve processes reacting do not differ in this respect from the axons of the myelinated fibers of mammals. The action of the excitants of the second class exhibits two peculiarities; the action is almost immediate, there is no latent period of several minutes as in mammals and in squid[1]; the fact that the nerve cells of the earthworm are unaffected by phenol, nicotin and creatin indicates a chemical organization different from that obtaining in the neurones of higher forms in which stimulation by these substances does take place.

108 (1690)

Observations on the specific exhaustion of cutaneous reactions.

By GEORGE M. MACKENZIE and LOUIS B. BALDWIN.

[*From the Medical Clinic of the Presbyterian Hospital,*
Columbia University, New York.]

Cutaneous reactions in hypersensitive individuals are of two quite distinct types. The reactions observed in patients with hay fever or asthma and after sensitization by foreign serum develop in a few minutes, consist essentially of a wheal and erythema, and fade out completely in one to two hours. The skin then appears normal. There is no visible evidence of cell destruction. Such reactions may be obtained with extracts of pollen, animal dandruff and feathers, with food proteins, with foreign serum, and occasionally with bacterial or other proteins.

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[1] Moore, A. R., *J. Gen. Physiol.*, 1919, i, 505.

This type of cutaneous reaction has little in common with the local cutaneous reactions to tuberculin, typhoidin, luetin, and mallein. Here the reaction does not usually develop for 12 to 24 hours; it is characterized by induration and persistent signs of inflammation, requires many days to fade out entirely and clearly involves cell destruction. Zinsser[1] has recently shown that the local tuberculin reaction in the guinea-pig is independent of the development of a state of anaphylaxis and it is highly probable that the same holds true for the reactions of this type produced by other substances of bacterial origin.

Although it has commonly been assumed that the immediate skin reactions with urticaria-like lesions are manifestations of anaphylaxis, it has not been demonstrated that the mechanism consists of an antigen-antibody reaction. Similar reactions may sometimes be obtained with non-antigenic substances such as aspirin, salicylates and quinine, and there are a few substances, notably histamine, morphine and pituitrin, which produce this type of reaction in normal individuals. It appears to be essentially a vascular phenomenon with localized edema.

In an effort to determine the nature of these urticaria-like skin reactions we have studied their exhaustibility by a simple procedure in five hypersensitive patients, the subjects of hay fever or bronchial asthma. Our results indicate that the reactivity of the skin may readily be abolished in the area involved in the reaction. This exhaustion has been accomplished with such biologically different substances as egg white, extracts of ragweed and chicken feathers, the proteins of almond, pea, oat and wheat. In the experiments in which the cutaneous method of eliciting the reaction was employed, the exhaustion was usually not complete until the reaction had been repeated five or six times on the same site, at intervals of one or two hours. When the intracutaneous method was employed and the protein solution injected into the skin, the rapidity with which the local exhaustion can be accomplished was found to vary with the concentration of the protein solution. With a 1–10 dilution of egg white, a single test done on a child of six with bronchial asthma and hypersensitiveness to egg, completely abolished the skin reactivity at the site of the reaction.

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[1] Zinsser, H., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 123.

The duration of the exhaustion has likewise been found to be dependent to some extent upon the concentration of the protein solution and the method employed for producing the reaction. With the 1–10 egg white solution and the intracutaneous method, the reactivity of the child's skin was completely abolished for three days and partially abolished at the site of the test for five days. In other instances with weaker solutions, the exhaustion did not persist more than twenty-four hours.

The extent of the area in which the reactivity is abolished is strictly limited to the site of the reaction. The area actually occupied by the wheal becomes completely exhausted, the area of the erythema partially so and beyond this the skin reacts as strongly as at a fresh site.

When two substances to which the individual gives positive reactions are simultaneously applied to the same site, the reaction is no stronger than that produced by the substances applied singly. There does not appear, therefore, to be any summation of effect.

Observations on the specificity of the exhaustion are not yet complete, but they suggest that there is a strict specificity for substances biologically unrelated and something similar to group reactions for substances closely related biologically. A patient reacting to ragweed and chicken feather showed a specific exhaustion, the exhausted ragweed site reacting as strongly to chicken feather as a fresh site and vice versa. With another patient, the specificity of the exhaustion was found to hold true for the proteins of almond and pea, while in a third patient giving positive reactions to wheat and oat, it was found that the exhausted wheat site reacted less strongly to oat than a fresh site.

As a control series of observations, we have attempted in six patients to abolish the cutaneous reactions to histamine. It is, of course, well known that the application of histamine in solution to a small cut or scarification in the skin produces an urticarial lesion quite similar in appearance to the reactions of individuals hypersensitive to pollen, dandruff and feather extracts. Sollman[1] has reported observations on the effect upon the skin reactivity of repeatedly applying histamine to the same site. In agreement with his results, we have found that the histamine skin reaction is not exhaustible—on the contrary, it progressively increases with each subsequent application on the same area. Since histamine is non-antigenic, the non-specific skin reaction produced by it can not be dependent upon an antigen-antibody reaction. To us, it seems significant that this non-antigenic substance produces a local reaction which is not exhaustible, while the antigenic substances which we have used in the skin reactions on hypersensitive patients produce a reaction which may easily be completely exhausted.

Since our observations demonstrate that it is possible to abolish locally the reactivity of the skin, it seemed possible that by local application of pollen or dandruff extracts to the mucous membrane of the nose and throat of patients with allergic rhinitis, the reactivity might here also be abolished. We have not as yet carried far enough this therapeutic application of our observations to draw conclusions, but the results, so far as they go, are very satisfactory.

109 (1691)

Preliminary report on a staphylococcus bacteriophage.

By **ANDRÉ GRATIA.**

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

In 1915, two years before the discovery of d'Hérelle, Twort described the following phenomenon.[2] If glycerinated calf vaccinia is streaked on agar slants, a certain number of the micrococcus colonies which grow become glassy and transparent, and degenerate into a granular material which cannot be subcultured and which, even when diluted 1,000,000 times and filtered, gives rise to the same degeneration when added to a normal culture of micrococci, and so on indefinitely.

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[1] Sollmann, T., and Pilcher, J. D., *Jour. Pharm. and Exp. Ther.*, 1919, ix, 309.

[2] *The Lancet*, 1915, ii, 1241.

Because of the similarity between these observations and the phenomenon of d'Hérelle, the following experiments were undertaken with the hope of obtaining from vaccinia a typical bacteriophage for staphylococci.

Several agar slants were seeded with untreated fresh green vaccinia pulp. The growth, consisting of *Staphylococcus albus*, *Staphylococcus aureus*, and *B. coli*, looked normal in all tubes but one, in which a few small, clarified areas were found.

Filtrates obtained from subcultures of these clarified spots were found to possess a marked inhibiting and dissolving action on the growth of staphylococci, and this lysis could be carried on indefinitely from one culture to another.

Staphylococcus is, therefore, the first Gram-positive bacterium for which an observation of transmissible autolysis has been made. Attempt to extend the lysis to other cocci has thus far been unsuccessful.

As has already been observed for other species, great variations in sensitiveness exist not only among different strains of staphylococci—certain ones remaining unaffected—but also between different organisms of a single strain, a few individuals usually being able to resist solution.

If a partially dissolved culture of staphylococcus is streaked on agar plates, it is found that in the first streak, where the lytic broth is spread abundantly together with the cocci which are still alive, the growth is poor, irregular and glassy. In the subsequent streaks, the colonies are less irregular, and finally become normally round and opaque. A round colony, transplanted, gives only round colonies, while an irregular colony gives colonies which, irregular in the first streaks, become less irregular and finally round in the following streaks.

In agreement with the observations described by Kuttner[1] for typhoid bacilli, and with our own observations on *B. coli*, an irregular colony was found to be lysogenic, a regular colony nonlysogenic.

In the course of our previous studies on coagulation of blood we observed a coagulant effect exerted by staphylococcus on all kinds of non-spontaneously-coagulable blood plasma (oxalated, citrated, salted, hirudinized, filtered plasma, etc.).[1] We have now observed that sterile filtrates of the above mentioned dissolved cultures of staphylococci possess the same property.

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[1] PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 158.

110 (1692)

On the preparation of a soluble protein extract from soy beans.

By **SELMAN A. WAKSMAN.**

[*From the New Jersey Agricultural Experiment Station, New Brunswick, N. J.*]

The average protein content of the soy bean is 36.5 per cent. while that of the soy bean cake is about 41 per cent. The protein of the soy bean has all the amino acids necessary for nutrition and it is claimed to be as valuable as the casein of milk. The proteins of the soy bean, unlike those of other leguminous plants thus far investigated, were found, by Osborne and Mendel, to be adequate for promoting normal growth. In addition to the high protein content, the soy bean is found to contain an adequate amount of water-soluble vitamine and some essential fat-soluble vitamine. These factors combined with low cost make the soy bean of unique significance as a food.

In working with proteolytic enzymes of fungi, the author found some organisms that are able to develop active enzymes which readily hydrolize the soy bean proteins and make them soluble. By using the ground soy bean cake as a substratum for the growth of the proteolytic fungus, then, at the end of the proper incubation period, which is usually about 36 hours, adding water to the mass and allowing the enzyme present in the mycelium to act upon the soy bean proteins, over 50 per cent of the proteins are found to go into solution. This solution when concentrated gives a material containing 45 or more per cent of soluble proteins and protein degradation products and is quite rich in vitamines. This extract can be very cheaply prepared and it may take the place, when properly modified by the addition of necessary salts, of meat extract and other digested meats in infant feeding and, since the soy bean contains very little carbohydrate and even the small amounts present are used up by the fungus, in the process of development, for energy purposes, the extract is practically free from sugars and can be introduced into diabetic cookery.

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[1] *Compt. rend. Soc. Biol.*, 1919, lxxxii, 1245, 1247, 1393; 1920, lxxxiii, 649, 584, 585, 649.

111 (1693)

Specific immunological reactions of Bence-Jones proteins.

By **S. BAYNE-JONES** and **D. WRIGHT WILSON.**

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A Bence-Jones protein which crystallized spontaneously from the urine of a patient (R.) at the Mayo Clinic in 1920 furnished material unusually well suited to the investigation of some of the problems associated with Bence-Jones proteinuria. With this specimen of the protein, which was purified by recrystallization, immunological studies were undertaken to discover, if possible, (1) a difference between various specimens of Bence-Jones protein, and (2) a difference between Bence-Jones protein and the proteins of human blood serum.

In the past, the few immunological studies on Bence-Jones protein have been directed solely toward the differentiation of human serum proteins from Bence-Jones protein. With the exception of Massini (1911), who was able to show by complement fixation tests specific distinctions between human serum and Bence-Jones protein in different zones of dilution, these investigations have apparently indicated that Bence-Jones protein and human serum proteins are immunologically indistinguishable. As in Abderhalden's (1905) experiment, these results have been attributable undoubtedly to the use of mixtures of proteins. Recently, Hektoen (1921) has published a preliminary note on his experiments which prove that by the absorption of precipitins specific reactions can be obtained even when mixtures are used, which sharply differentiate Bence-Jones protein from the proteins of human blood serum.

The work to be reported here was completed before the appearance of Hektoen's paper.

Studies were made upon 13 preparations of Bence-Jones protein from various sources and prepared in various ways. Comparative studies of these preparations and of human serum were carried out by the use of precipitin, complement fixation and anaphylactic reactions.

Precipitin Reactions.—Rabbits were injected intravenously with solutions of the crystalline Bence-Jones protein, an ammonium-sulphate preparation of a non-crystallizable specimen of this protein from another source, and with human serum. After immunization, the serum of these animals contained precipitins which flocculated the homologous antigens in high dilutions. In the series of comparative precipitation tests, all the solutions of Bence-Jones proteins were used in a concentration of 4 per cent., and from this dilutions were made. The hydrogen-ion concentration of the fluids and the environmental conditions were uniform. Readings of precipitation were made by the ring tests after 1 hour at room temperature, and again after 24 hours at 37° C. The antiserum to the crystalline Bence-Jones protein precipitated a 1 to 1,000,000 dilution of a 4 per cent. solution of this preparation, affected only slightly or not at all the other preparations from other sources, and gave no trace of precipitate with human serum. By the use of this purified preparation of Bence-Jones protein, therefore, it was possible to show at once that there are differences between various Bence-Jones proteins as regards their precipitability by an antiserum to one substance of this class, and that there is a sharp immunological distinction between Bence-Jones protein and human serum. Corroboration of these findings came next through the use of the antiserum to the non-crystallizable Bence-Jones protein. This serum did not precipitate the crystalline Bence-Jones protein, variously affected the solutions of the other preparations, and gave a precipitate with human serum at 1 to 1000. Antihuman serum did not precipitate the solution of the crystalline Bence-Jones protein, but gave precipitates with the urines of several patients with Bence-Jones proteinuria, and with all of the salted-out preparations of Bence-Jones protein.

Complement Fixation Reactions.—The usual antisheep amboceptor system with guinea pig complement was used and all controls were made with quantities of solutions double those used in the tests. In general, complement fixation tests like the precipitin reactions, showed differences between the various preparations of Bence-Jones protein, a cross-reaction between human serum and the salted-out specimens of non-crystallizable Bence-Jones protein, and a complete difference between the crystalline Bence-Jones protein and blood-serum.

Anaphylactic Reactions.—It was difficult to sensitize guinea pigs to the crystalline Bence-Jones protein, though not to the other preparations, indicating again an antigenic difference in that respect. Guinea pigs were sensitized actively by the intravenous injection of 0.25 c.c. of a 6 per cent. solution of the various Bence-Jones proteins, and by 0.25 c.c. of human serum. Some animals were passively sensitized by the intraperitoneal injection of the antiserum to the crystalline preparation. Three weeks after the first injection of protein or human serum in the series of actively sensitized animals, these guinea pigs were tested in two ways for specific sensitivity. The reaction of the animal as a whole was used when the intoxicating dose was given intravenously or intraperitoneally, and the method of Schultz and Dale was used with the uterine horns of the guinea pigs to provide graphic records of the experiments. These reactions also demonstrated (1) differences between the various Bence-Jones proteins, (2) a mixture of human serum proteins and Bence-Jones proteins in the preparations made in the attempt to salt-out Bence-Jones protein from the urine, and (3) complete difference between the crystalline Bence-Jones protein and human serum. (Demonstration of charts of precipitin and anaphylactic reactions.)

112 (1694)

On the influence of tissue enzymes on the bacteriophage principle.

By **ANN G. KUTTNER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

I have previously reported before this Society the isolation of a lytic principle active against typhoid and dysentery bacilli obtained by the d'Hérelle technique from the filtrate of a stool from a typhoid convalescent. The fact that the lytic agent could be transmitted apparently indefinitely in series and that it was only active against vigorously growing bacteria, suggested that the lytic agent might be derived from the bacterial cell itself. My next step was to try to produce a lytic principle from the typhoid bacilli without any interaction with the living animal body.

I proceeded on a theory first suggested by d'Hérelle, but discarded by him, namely, that the so-called phenomenon of d'Hérelle might be due either to an activation of the natural autolysin present in all bacteria, or to the removal of autolysin-inhibiting substance. Once this natural autolysin was liberated, it could in turn liberate an active autolysin from the next generation of bacteria and so on indefinitely.

It seemed possible from the work of Twort and other observers, such as Cantacuzène and Marie, and from the more recent papers of Turro, that tissue extracts might play a part in starting the activities of the autolysin.

I want to report briefly on some preliminary results I have obtained by the action of tissue extracts on typhoid bacilli. Up to the present time, I have obtained lysis of typhoid bacilli, transmittable in series by the action of extracts of two different tissues, namely: small intestine and liver. Both tissues were derived from guinea pigs. In the case of the small intestine, three small intestines from normal guinea pigs were pooled, washed and minced. Without drying the tissue was divided and extracted in different strengths of glycerine. After 11 days extraction in 50 per cent. glycerine at 37° C. some of the supernatant fluid was centrifuged and filtered through a Berkfeld. The addition of a small amount of this filtrate to a young turbid typhoid culture produced a slight amount of clearing as compared with the control. A loop from this tube was plated and then heated at 55° C. The plate showed regular and irregular colonies. The broth fishings of the irregular colonies carry the lytic principle and typhoid bacilli can be dissolved in series starting with a broth fishing of one of these irregular colonies. It has also been possible to transmit the lytic principle from the first tube after heating to kill the resistant typhoid bacilli, and I have obtained lysis of typhoid bacilli in seventh generation removed from the tissue extract. Small intestine extracted with 25 per cent. glycerine has given similar results. Glycerine extracts of the large intestine and of muscle tissue have so far given negative results.

I prepared the liver extract according to the method used by Turro in preparing tissue extracts. Turro has reported that extracts of leucocytes, muscle tissue, kidneys, pancreas, thyroid, etc., digest bacterial protein. He has worked particularly with anthrax bacilli, but also with cholera and typhoid. He does not in any of the papers that he has published up to this time, connect his results with the phenomenon of d'Hérelle, and does not show that the dissolved bacteria can dissolve new cultures. He states that no special ferment derived exclusively from polynuclear leucocytes is necessary to digest bacteria, but that all tissue cells probably contain such ferments.

The liver extract was prepared in the following way: The liver from a normal guinea pig was minced, shaken up with acetone, dried in vacuo and pulverized. To approximately one gram of liver powder, 20 c.c. of salt were added. To one tube, 40 drops of chloroform were then added and to the other, a small amount of sodium fluoride. The tubes were placed in the incubator for 14 hours. The tube with chloroform was sterile, the tube to which the sodium fluoride was added, was contaminated, both were centrifuged and the latter was filtered through a Berkfeld.

Both these liver extracts dissolve cultures of typhoid bacill and the lytic principle can be transmitted in series from the dissolved culture. The 6th generation from the liver extract has now been reached and the lytic action has increased both in the degree of clearing and in the rapidity of the action. Cultures are not sterilized completely and the two types of colonies, one, the bearer of lytic principle, develop on plating. Control experiments to determine whether it is the action of the glycerine on the bacteria that produce the lysis have been negative. Similar control experiments with chloroform and sodium fluoride have up to the present time not produced a transmittable lytic principle.

These tissue extracts do not appear to be specific, but the range of their action has not yet been determined. Both the intestinal extract and the liver extract are active against several different strains of typhoid so that the results cannot be attributed to the idiosyncrasies of one strain. The strains have been plated out repeatedly to see if irregular colonies carrying the lytic principle could be obtained from the normal culture, but up to the present time no lytic principle has been isolated without the interaction of tissue enzymes with the typhoid bacilli.

113 (1695)

Growth-determining substances in bacteriological culture media.

By J. HOWARD MUELLER.

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Some months ago a report was made of a series of experiments based on the observation that, while a peptone-free meat infusion broth would produce abundant growth of hemolytic streptococci, short boiling with charcoal removed this property entirely. The addition of commercial peptone or of a sulphuric acid hydrolysate of certain proteins, such as casein or meat, reactivated the charcoal-treated infusion and heavy cultures of streptococci could be obtained on the mixture, while neither one alone gave the slightest trace of growth. It was shown that the activating material in the protein hydrolysate was precipitated by mercuric sulphate, and that it had not been possible to identify it with any of the amino acids known to be precipitated by this reagent either alone or in combination. It is the purpose of the present communication to describe the further purification of this activating material.

Much of the work has been done using a commercial preparation called "aminoids" in place of an acid hydrolysate of casein. This consists of an enzyme digest of milk proteins continued until the product is biuret free. It has been used simply as an economy of time since in handling large quantities the acid hydrolysis is somewhat cumbersome. Every step in the separation, however, has been checked on an acid hydrolysate and it is believed that there is no essential difference in the factors involved.

In attempting to separate the active material from the mercuric sulphate precipitate, fractional precipitation with the same reagent was tried. This led to the discovery that there were two factors in the precipitate, both of which were necessary to reactivate the charcoal-treated infusion. One of these must be carried down by adsorption in the original mercuric sulphate precipitate, or else its solubility is influenced by the presence of other substances, for it is not reprecipitated, to any extent, from the mixture by the addition of mercuric sulphate. If the filtrate from the first precipitation is tested for this factor, it is found to be present in moderate concentration, although apparently less than in the precipitate.

There is some difficulty in making this separation quantitative with mercuric sulphate, and a more convenient reagent was found in silver sulphate and baryta. The original mercury precipitate is freed from mercury by H_2S, and after boiling out the H_2S and cooling, silver sulphate solution is added in slight excess, and barium hydroxide solution to moderately alkaline reaction to litmus. The precipitate, which is freed from silver by H_2S, contains histidine and considerable brown sticky material, in addition to an active fraction which may be briefly referred to as "X." If necessary, the second factor, or "Y," most of which remains in the silver filtrate, may be removed more completely by reprecipitating with silver sulphate and baryta.

In several experiments it was found possible to precipitate the histidine and the pigment with phosphotungstic acid, leaving the active "X" in the filtrate. Evaporation to dryness after removing the phosphotungstic acid, yielded a semi-crystalline material, but also destroyed the activity of this fraction. These experiments were made with a single solution of phosphotungstic acid. With all the subsequent preparations of the reagent complete loss of activity has resulted during the precipitation of the crude "X" fraction, and neither the precipitate nor the filtrate nor both together have given growth. It is possible that oxidation may explain this change in properties, and further work must be done in attempting the isolation of this factor.

The silver sulphate filtrate, or "Y" fraction, does not give a precipitate with phosphotungstic acid. Mercuric sulphate throws down a rather abundant precipitate, composed probably of tryptophane and tyrosine. The filtrate from this precipitate contains the "Y" factor. This can be concentrated on the water bath to a small bulk, filtered from tyrosine after standing overnight on ice, and concentrated further with the addition of alcohol, to beginning crystallization. On standing, crystals separate consisting of microscopic spheres apparently made up of finely interwoven needles. These crystals are exceedingly soluble in water, and quite soluble even in 70 to 80 per cent. alcohol, and there is considerable loss on recrystallization. In one preparation the yield after one recrystallization from strong alcohol was 0.012 g. from 200 g. aminoids. Growth was given with 0.000,01 g. of these crystals in 25 c.c. of media. Further recrystallization apparently either eliminates the active factor from the main bulk of crystals, or else alters the chemical nature of the substance, since growth becomes very slow and scanty. The mother liquors, still containing considerable quantities of crystalline material, together with some amorphous brown substance, likewise show diminished activity, so that there is probably in the case of the "Y" factor, as with the "X," a certain amount of lability as the preparations become purer. I do not wish to state definitely at this time the belief that the crystals just described are in fact the active "Y" material, and further work with larger quantities must be done. There is, indeed, no assurance that the crystals are pure because of the difficulty in recrystallizing caused by the high solubility.

After two recrystallizations, the crystals when dried at 100° are light and powdery. They give a moderately strong reaction with Folin's phenol reagent, but no color with the nitro-prusside test. Nitrogen is present to the amount of 10.6 per cent. by the micro-kjeldahl method, and qualitative tests for sulphur are positive after fusion with sodium, but the lead-acetate test on boiling with NaOH is negative. Phosphorus and halogens are not found. Sufficient material for complete quantitative analysis has not yet been prepared.

In the first report on this work, it was stated that the hydrolysates of certain proteins, such as gelatine, were not capable of reactivating the infusion. At that time, it was not recognized that two substances were involved, and in the light of that fact, a further investigation should be made as to whether those proteins are deficient in both the "X" and the "Y" factors, or whether one may occur without the other. A few preliminary tests have indicated that gelatine and egg proteins contain the "Y" and are deficient only in "X," but the results were not clean cut, and it is possible that other factors came in. Lack of time has prevented the extending of these observations.

Without more definite knowledge of the chemical nature of these two substances, speculation as to the manner in which they induce growth of the streptococci does not seem warranted. There is no means at present of knowing whether they act as "building-stones" in supplying some necessary grouping in the synthesis of the bacterial protein, or whether they simply initiate or accelerate some essential vital process. Perhaps, in the light of much recent work dealing with the effect of vitamines on bacterial and yeast growth, it is not unwarranted to believe that still other phases of animal metabolism may be cleared up in part through work on the metabolism of lower forms of life. In the case of the study of the streptococcus, there are at least three factors, still unidentified, which determine growth; namely, some substance in the charcoal-treated infusion, the "X" fraction, and the "Y" fraction. It is by no means felt that all or any of these factors if isolated, will prove to be new physiological compounds, but if such should be the case, one must believe, in order to explain their occurrence in meat, milk, etc., that they also play a part in animal metabolism.

114 (1696)

The supposed relation between alkalosis and tetany and similar conditions.

By **ISIDOR GREENWALD.**

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Examination of the work of Wilson, Stearns and Thurlow[1] shows that their conception of an "alkalosis" as one of the consequences of parathyroidectomy rests essentially upon the supposed increases in the oxygen saturation of the blood at definite oxygen tensions. Wilson, Stearns and Thurlow used Barcroft's[2] formula

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[1] Wilson, D. W., Stearns, T., and Thurlow, M. de G., *Journal of Biological Chemistry*, 1915, xxiii, 89.

$$\frac{y}{100} = \frac{Kx^n}{1 - Kx^n},$$

in which y is the percentage saturation of the blood with oxygen, x is the oxygen tension in millimeters of mercury, and n is a constant to which Barcroft assigned the value 2.5 for human blood. Wilson, Stearns and Thurlow assumed the same value for dog blood although calculations from Barcroft's value for dog blood (Barcroft, page 50) show that, in this case, the value of n was not 2.5, but approximately 2.2. Barcroft worked at 40 mm. CO_2, Wilson, Stearns and Thurlow at 0 mm. CO_2. According to Barcroft, this difference does not affect the value of n in human blood. But calculating from the figures of Wilson, Stearns and Thurlow, which differ considerably from one another, the value of n is found to be not 2.5, nor 2.2, but approximately 1.5. Using this value of n in recalculating the value of K from the data of Wilson, Stearns and Thurlow it is found that this is not regularly greater in the blood of parathyroidectomized dogs than in the blood of normal dogs. The significance of such changes in percentage oxygen saturation as do occur is obscure. Many factors, other than change in reaction or in alkaline reserve may be responsible. Barcroft's figures (Barcroft, page 62) indicate a specific effect of phosphates in increasing the value of K and decreasing that of n. Moreover, any unrecognized decrease in n will increase the apparent value of K. A few calculations indicate that the retention of phosphate and acumulation thereof in the blood after parathyroidectomy may possibly be sufficient to account for any observed changes in percentage oxygen saturation without involving any change in reaction or in alkaline reserve. Hastings and Murray[3] have shown by their own work and by reference to the more recent literature that there is no direct evidence of either an increased alkalinity nor increased CO_2-capacity after parathyroidectomy.

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[2] Barcroft, J., "The Respiratory Function of the Blood," Cambridge, 1914.

[3] Hastings, A. B. and Murray, H. A., Jr., *Journal of Biological Chemistry*, 1921, xlvi, 233.

An attempt was made to induce symptoms resembling tetany by the intravenous injection of $NaHCO_3$ into dogs, under cocaine anesthesia. Convulsions could be produced in this manner. There was, also, a decrease in the hydrogen ion concentration of the blood and plasma, as measured with the potentiometer or by calculation from the bicarbonate and total CO_2 content. The change in reaction was never great and was less in convulsions than before the neuromuscular symptoms had become so marked. Rapid injection produced apnea, with death from respiratory failure, and not convulsions (Table I). The bicarbonate content was enormously increased, the values observed ranging from 162 to 226 volumes per cent. CO_2. At the reactions observed, such bicarbonate concentrations require high tensions of CO_2 in the alveolar air, a value as high as 161 mm. being calculated in one case from the observed total and bicarbonate CO_2. The convulsions could not be relieved by the injection of HCl, although the bicarbonate content was thereby much reduced, to even below the normal level. One dog received distilled water and another a calcium chloride solution. The convulsions continued in both cases but the life of the latter animal seemed to be prolonged somewhat. One dog received a mixture of bicarbonates containing K, Ca and Mg in approximately 0.1 the concentration, relative to the Na, that obtains in dog plasma. Another dog received in one vein a mixture of sodium and potassium bicarbonates and, in another vein, a mixture of calcium and magnesium chlorides. The amounts injected were so adjusted as keep the relations between Na, K, Ca and Mg the same as those obtaining in dog plasma. Both dogs developed convulsions, but the *former* attained a higher concentration of sodium in the plasma than was secured in any other experiment.

Comparison with the results of similar experiments with other sodium salts[1] indicates that the concentration of sodium in the plasma required to produce convulsions is approximately the same for sodium bicarbonate, chloride, phosphate or sulfate (Table II). Attention is called to the experiments of Hougardy[2] who injected sodium hydroxide and other alkalies into dogs and rabbits and to those of Scott[1] who injected sodium carbonate into decerbrate cats. Both obtained respiratory effects and the latter believed he obtained marked changes in the reaction of the blood but neither mention any sign of neuromuscular symptoms resembling tetany or convulsions. Hougardy found that the slightest excess of alkali killed his animals promptly.

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[1] Greenwald, I., *Journal of Pharmacology and Experimental Therapeutics*, 1918, xi, 281.

[2] Hougardy, A., *Archives international de Physiologie*, 1904, i, 17.

Scott employed smaller doses and a slower rate than were used in the present experiments and obtained much lower values for total CO_2 content of the blood than are here reported for plasma. But the changes in hydrogen ion concentration he reports are at least as great as those in the present experiments. The validity of his conclusions as to the specific function of the bicarbonate ion as a respiratory hormone rests entirely upon the accuracy of his determinations of the hydrogen ion concentration. From *a priori* considerations and from the results here reported it would appear that the dialysis colorimetric method employed by Scott gives too high P_H values particularly under the high CO_2 tensions obtaining after the injection of sodium carbonate. The significance of the bicarbonate ion as a respiratory hormone is, therefore, highly questionable.

Within the last few years there have been a number of reported cases, and probably a much larger number of unreported cases, of tetany appearing in patients after the intravenous injection of sodium bicarbonate for therapeutic purposes. A high CO_2 combining capacity, 80 volumes per cent. or more, has been observed and the phrase "alkalosis" has been accepted as explaining the appearance of the symptoms. Palmer, Salvesen and Jackson[2] regard it as dangerous to administer sodium bicarbonate by mouth in amounts greater than those required to produce the first significant change in the reaction of the urine. Such caution would appear to be needless. Patients with gastric hyperacidity may take sodium bicarbonate in amounts sufficient to make the urine alkaline for days at a time without any sign of tetany. The appearance of an alkaline urine is not a danger sign. Rather is it an indication that the kidneys are removing at least some of the excess alkali. A high CO_2-combining capacity, however, may be regarded as dangerous but not because of any change in reaction. A CO_2-capacity of 80 volumes per cent. would require an alveolar CO_2 tension of only about 60 mm., at $P_H = 7.4$. An adjustment of respiration to secure this would not seem to be beyond the body's powers. It appears more likely that the danger is that this high CO_2-capacity is due to a retention of sodium sufficiently great to disturb the normal kation equilibrium. As was indicated in a previous paper, the nature of the anion appears to be of significance as it affects the permeability of the tissue cells to the sodium. It may be that the sodium enters the cells and so poisons them or it may not enter and so bring about a change in the potential difference at the cell boundary.

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[1] Scott, R. W., *American Journal of Physiology*, 1917, xliv, 196; 1918, xlvii, 43.

[2] Palmer, W. W., Salvesen, H., and Jackson, H., Jr., *Journal of Biological Chemistry*, 1920, xlv, 101.

The alkalosis of hyperpnea remains to be considered. Calculations from the figures of Collip and Backus[1] indicate that hyperpnœa increased the P_H of the plasma by from 0.1 to 0.4, with an average of from 0.2 to 0.25, depending upon the method of calculation. The 15 experiments give exceedingly concordant results. However, there is one scource of error that may apply to all and that would give too high P_H values after hyperpnœa. After forced respiration, the usual period of holding the breath may not be sufficient to bring the alveolar air into equilibrium with the blood and the value for the CO_2 tension may therefore be too low. It is unfortunate that no determinations were made of the hydrogen ion concentration nor of the CO_2 *content* of the plasma. However, there must have been some change in reaction for the character of the urine changed, becoming more alkaline in spite of the diminished excretion of ammonia and increased excretion of phosphates. But it by no means follows that this change in reaction was, *per se*, the cause of the tetany-like symptoms observed. The changes in the urine indicate a very decided disturbance in the equilibrium between the various ions within the body.

Forced respiration, like repeated gastric lavage or pyloric obstruction, is a very effective method of removing acid from the body. The organism is not so well adapted to caring for this sort of disturbance as it is for the introduction of acid or alkali. Nevertheless, as Hastings, Murray and Murray[1] have shown, there is, at the most, an inconsiderable increase in the alkalinity of the plasma after pyloric obstruction. The CO_2 capacity is increased but not the alkalinity.

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[1] Collip, J. B., and Backus, P. L., *American Journal of Physiology*, 1920, li, 568.

It is interesting to note that whereas in the paper dealing with the effects of forced respiration, Collip and Backus accept the view that tetany is due to alkalosis, even going so far as to suggest that muscle "cramp" and ether spasm may be due to alkalosis, in a later paper dealing with the effects of the sub-arachnoid and intra-arterial injection of sodium bicarbonate and other electrolytes, Collip[2] emphasizes the disturbance in the kation

TABLE I.

Exp	P_H Before[3]		P_H After $NaHCO_3$[3]		Plasma CO_2 after $NaHCO_3$.				Remarks.[7] $k = 3.04 \quad 10^{-7}$ $\delta = 0.8$
	Blood	Plasma.	Blood	Plasma.	$NaHCO_3$ Per Cent.[4]	Total Per Cent.[5]	Tension (cal.) mm.[6]	Calcd P_H from $H = \frac{k\ CO_2}{\delta\ NaHCO_3}$	
4	7.06	7.40	7.06	7.15	162				No tremor or twitching. Death due to respiratory failure.
			7.43						Aërated blood.
5	7.02	7.37	7.37	7.45	183				Convulsions.
					162				Blood drawn after death in convulsions.
6		7.34	7.33	7.56	139				Convulsions.
			6.88		26				Blood drawn after death after injecting HCl.
7	7.01	7.33	7.50	7.51	177	191	122	7.51	Slight tremor.
			7.24	7.43	226	245	161	7.49	Convulsions.
					96	108	104	7.31	Blood drawn after death after injecting HCl.

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[1] Hastings, A. B., Murray, C. D., and Murray, H. A., Jr., *Journal of Biological Chemistry*, 1921, xlvi, 223.

[2] Collip, J. B., *American Journal of Physiology*, 1920, lii, 483.

[3] More recent experiments indicate that the anticoagulant used lowered the P_H values in blood by about 0.33 and in plasma by about 0.15.

[4] Van Slyke, D. D., Stillman, E., and Cullen, G. E., *Journal of Biological Chemistry*, 1919, xxxviii, 167.

[5] Van Slyke, D. D., *Journal of Biological Chemistry*, 1917, xxx, 347.

[6] Assuming solubility of CO_2 in plasma to be 0.9 that in H_2O.

[7] Walker, J., and Cormack, W., *Journal of Chemical Society*, 1900, lxxvii, 5.
Michaelis, L., and Rona, P., *Biochemische Zeitschrift*, 1914, lxvii, 182.
Evans, C. L., *Journal of Physiology*, 1920, liv, 353.

equilibrium, though he also ascribes a specific stimulating effect to the bicarbonate ion. To the present author, it seems that this specific effect may be due entirely to the effect of the anion in determining the permeability of the tissues to the kation.

TABLE II.

Ex.	Salt.	Concn.	Time.	Sodium.			Remarks.
				Per Kilo Body Weight		Per 100 cc. Plasma.	
		N.	min	Injec. Gr'ms	Retained Gr'ms		
1	$NaHCO_3$	0.9	38	0.694	0.493	442	Twitching.
			44	0.831	0.631	447	Convulsions.
5	$NaHCO_3$	0.9	40	0.747	0.730	436	Twitching.
2	$NaHCO_3$	0.9	43	0.783	0.684	514	Twitching. Injected soln. contained 0.1 equivalent of K, Mg and Ca.
3	$NaHCO_3$	0.9	40	0.793	0.709	457	Convulsions. Injected soln. contained 1.0 equivalent of K, Mg and Ca.
4	$NaHCO_3$	0.47	68	1.140	0.87	420	No twitching or tremor. Death due to respiratory failure.
5	NaCl	0.60[1]	35	1.45	1.30	493	Convulsions.
6	Na_2HPO_4 NaH_2PO_4	0.38[1]	33	0.70	0.62	451	No twitching. Death due to respiratory failure.
8	Na_2SO_4	0.25[1]	111	1.29	0.82	480	Twitching.

115 (1697)

The peptolytic enzymes of hemolytic streptococci; methods.

By **RANDOLPH WEST** and **FRANKLIN A. STEVENS.**

[*From the Medical Clinic of the Presbyterian Hospital, Columbia University, New York City.*]

During the study of the virulence of hemolytic streptococci, it has been necessary to understand the action of the cocci on certain protein fractions. On account of the structure of these bacteria, the methods required to obtain the active proteolytic substance from the bacterial cell, and to accomplish the sterilization of the solution containing the enzyme were at first consuming and laborious. With the procedure outlined active solutions may be obtained without difficulty. All procedures were sterile. Beef infusion has been used as a base for the media. It has been prepared in the usual way except for the addition of 0.1 per cent. dextrose. After the ingredients were dissolved in the infusion, the broth was titrated to P_H 8.5 or 9.0 and boiled until the phosphates were precipitated out. It was then filtered, adjusted to P_H 8.0 and autoclaved. Precipitation during the sterilization was avoided by the preliminary boiling in alkaline solution. The glucose was apparently not decomposed to an extent sufficient to interfere with the growth of the bacteria. The acidity developed in the growth of the culture (about P_H 6.5) causes the spontaneous agglutination of the streptococci, yet it is not detrimental to the production or the life of the enzyme.

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[1] These figures represent the average concentration. More dilute solutions were employed at first and more concentrated ones later. See protocols in Greenwald, *Journal of Pharmacology and Experimental Therapeutics*, 1918, xi, 281.

Flasks of 6 L. volume were seeded with a broth culture of Strain Py 3, a beta type streptococcus of human origin. After 12 hours, the clear supernatant broth was decanted by siphon and the agglutinated streptococci were centrifuged, washed repeatedly in physiological salt solution and resuspended in 15 c.c. of $M/15$ phosphate of P_H 7.0. They were then dried in an agate mortar in vacuum after the addition of 2 grams of powdered glass. Grinding was carried out until there was a minimum amount of Gram positive material in the smear. When they were well macerated, the bacterial mass was taken up in 50 c.c. of distilled water, and allowed to stand under toluol at 10 degrees C. for 12 to 24 hours. The fluid pipetted from beneath the layer of toluol was centrifuged. It contained the active enzyme and was sterile. The sediment contained no demonstrable peptolytic substance.

The substrate used in the experiments was a 1 per cent. solution of peptone prepared especially for this work by Fairchild Bros. and Foster; this peptone contained 12.5 per cent. nitrogen of which approximately one fifth could be determined as amino nitrogen according to Van Slyke. The solutions of peptone were sterilized in the Arnold sterilizer. To determine the effects of H-ion concentration, heat and enzyme concentration series of 20 c.c. volumetric flasks were prepared with the enzyme, $M/15$ buffer mixtures (phosphate or citrate) and sufficient substrate to bring the final concentration to 1.0 per cent. Duplicate flasks with boiled enzyme served as a control for each determination of the series. After incubation at 37 degrees for 48 hours, the sterility of the experiments was proven by broth cultures and the increase in amino N determined by the micro apparatus.

The increase in amino nitrogen with various enzyme preparations has varied from 14 to 26 mg. per cent. By varying the H-ion concentration of the solutions a maximum activity was found at P_H 7.0 with a decrease on both the alkaline and acid sides of neutrality. The acid endpoint of activity was about P_H 4.5. It was further found that heat destroyed the enzyme very readily. At a temperature of 56° C. for ten minutes in the absence of substrate, the enzyme deteriorated to such an extent that only a few mg. of amino nitrogen were obtained by its action. Higher temperatures completely destroyed it. Concentration curves at the optimum H-ion concentration approximated the usual curves for enzymes.

116 (1698)

A study of the Wassermann reaction in one hundred and forty cases of diabetes mellitus.

By **JACOB ROSENBLOOM.**

In a study of one hundred and forty cases of diabetes mellitus, a positive Wassermann reaction was found in sixteen cases. Eight of these sixteen cases presented signs of tertiary syphilis. These eight cases were treated for the existing syphilis. There was no increase in tolerance for carbohydrate following the treatment. This may be due to the fact that the fibrosis of the pancreas produced by the syphilis is not changed by the treatment.

117 (1699)

Blood pressure studies in one hundred and forty cases of diabetes mellitus.

By **JACOB ROSENBLOOM.**

Blood pressure estimations were carried out on one hundred and forty cases of diabetes for varying lengths of time. Some of these cases have been studied for a period as long as ten years. On the basis of these studies it may be concluded that the blood pressure in uncomplicated diabetes is normal or slightly under normal. In every case of elevated blood pressure in this series I found complications such as aortitis, arteriosclerosis, nephritis, cardiac hypertrophy and aortic endocarditis. In conditions of acidosis the blood pressure falls. The presence or absence of hyperglycemia had no effect on the blood pressure.

118 (1700)

The antigenic properties of ragweed pollen.

By **JULIA T. PARKER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

Although most of the evidence would lead one to believe that pollens are antigenic, there are a few experimenters who still hold to a contrary opinion because they have been unable to produce antibodies to pollens. The question is of interest in its relation to hay fever. If pollens are antigenic, the anaphylactic nature of hay fever may be regarded as at least a possible explanation of the phenomenon. As pollens contain protein, although in small amounts, it would naturally be assumed that when sufficiently large quantities of pollen have been injected and delicate enough tests performed, antibodies could be shown to be present.

Although we have only two experiments to report, our results are so convincing that we feel that a definite conclusion is justifiable. These results were obtained by testing the isolated uteri of sensitized guinea pigs by the Dale Method.

EXPERIMENT I.

Three female guinea pigs were sensitized with pollen extract prepared as follows: 500 mg. of ground Mulford high ragweed pollen were shaken in a bottle in 200 c.c. of 0.04 per cent. NaOH in physiological salt solution for at least an hour on three successive days. This material was centrifugalized and the clear supernatant fluid, which gave the Millon and zanthroproteic protein tests, was injected intraperitoneally into the three guinea pigs. 70 c.c.

in all were given to each guinea pig during the course of seven weeks. The last injection was given November 29, 1920. We attach considerable importance to the method of sensitization which was similar to that found best in this laboratory in the case of bacterial proteins. We did not rely on one or two sensitizing injections, but injected every day for several weeks.

The uteri of the three treated guinea pigs were tested by the Dale method on December 22 and 23, 1920. The uteri of two normal pigs were also tested. 3 c.c. of the pollen extract when added to the bath of 200 c.c. Ringer's solution had no effect on the normal uteri; while 1 or 2 c.c. of the same extract produced marked contraction and prolonged spasm of the sensitized uteri. In one instance, 3 c.c. completely desensitized one horn of a sensitized uterus to the further instillation of 3 c.c. of pollen extract into the bath.

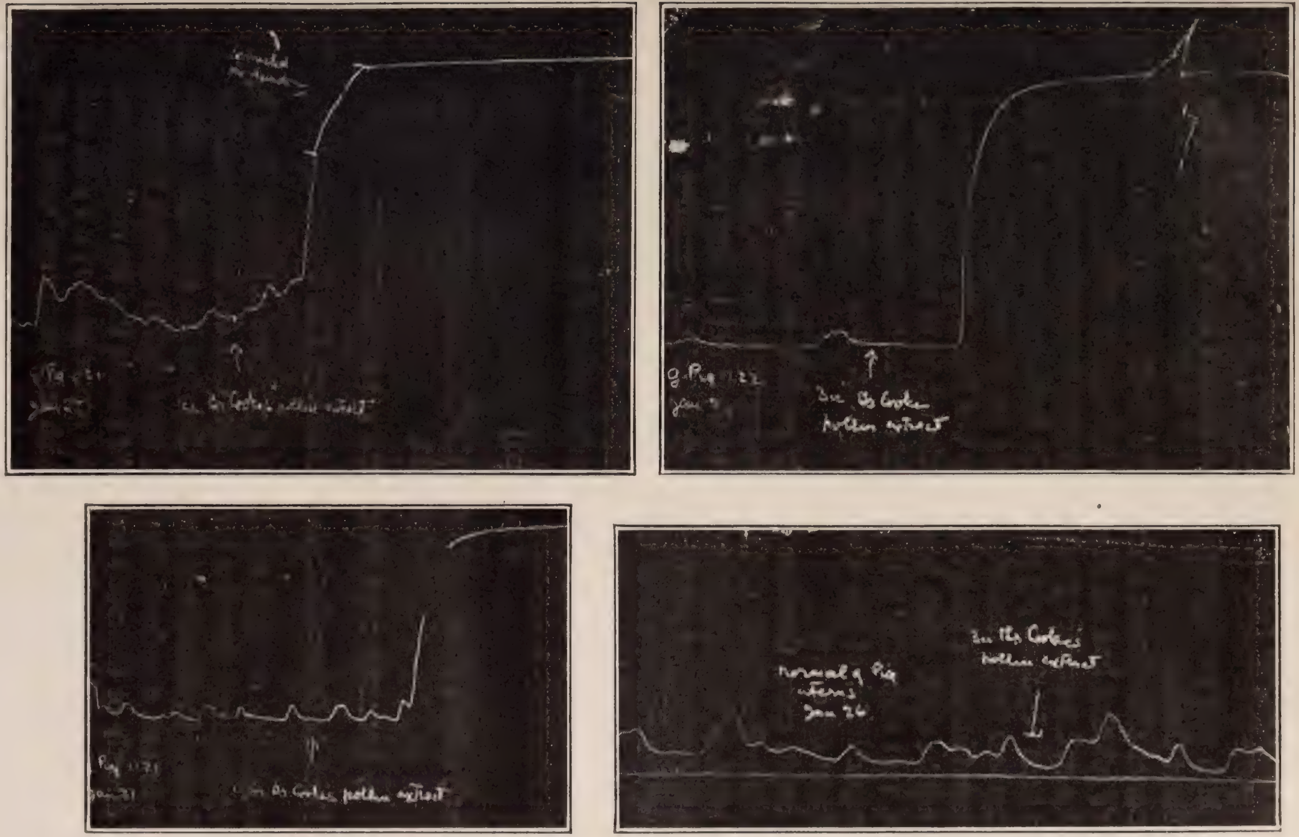
The extract added to the bath in the Experiment I was prepared by extracting the pollen with 0.02 per cent. Na_2CO_3, instead of with 0.04 per cent. NaOH.

The graphic record of this experiment is omitted because it is in principle exactly like the record of Experiment II, which is given below.

In view of the fact that clinical observers, Cooke and Vander Veer, were obtaining skin reactions with pollen extracts, the antigenic nature of which they, as well as Coca, denied, it was natural to think of the possibility that our preparations might be fundamentally different from the ones they were using. It was of course of great practical importance to determine whether or not their preparation would fail to show antigenic properties if tested by our methods. Accordingly, we obtained from Dr. Cooke a specimen of the preparation of pollen actually used by them on patients, and the sensitiveness of the guinea pigs sensitized by the method described above, was then tested with this material.

EXPERIMENT II.

Two female guinea pigs, No. 1121 and No. 1122 were sensitized with Mulford high ragweed pollen extracted with 0.02 per cent. Na_2CO_3 solution. 36 c.c. in all, in doses of 2 c.c., were given to each guinea pig in the course of 17 days. The last injection was given on December 31, 1920.



Guinea Pigs No. 1121 and No. 1122, and a normal guinea pig. Two guinea pigs, No. 1121 and No. 1122, injected intraperitoneally every day with an 0.02 per cent. sodium carbonate extract of Mulford high rag-weed pollen in physiological salt solution. Total amount injected, 36 c.c. Last injection received December 31st, 1920. Uteri of guinea pigs No. 1121 and No. 1122 and the uterus of a normal guinea pig tested with Dr. Cooke's pollen extract on January 26th and 27th. This was a sodium carbonate extract of both high and low rag-weed pollen. Uterus of guinea pig No. 1122 was found desensitized to 3 c.c. of the sodium carbonate high rag-weed extract 29 minutes after Dr. Cooke's extract had been instilled in the bath. Volume of bath = 200 c.c.

The uteri of the 2 sensitized pigs and one normal pig were tested by the Dale method on January 26 and 27, 1921, with some mixed high and low ragweed pollen extract kindly given us by Dr. Robert Cooke.

3 c.c. of this extract when added to the bath of 200 c.c. of Ringer's solution had no effect on the normal uterus. 3 c.c. of the same pollen extract produced marked contraction and spasm on one horn of guinea pig uterus No. 1122, and 29 minutes later this horn was found desensitized to 3 c.c. of our Na_2CO_3 extract. The second horn of uterus No. 1122 was found very irritable and could not be used. Both horns of the uterus of sensitized guinea pig No. 1121 responded with marked contraction to 1 c.c. and 0.5 c.c., respectively, of Dr. Cooke's extract. For records of this experiment see curve.

It would seem, therefore ,hardly possible to doubt that ragweed pollen is antigenic and that the negative results obtained by other workers were probably due to their not having employed adequate methods of sensitization or sufficiently sensitive tests. We may, therefore, assert the antigenic nature of ragweed extracts, without wishing at the present time to draw any theoretical conclusions as to the anaphylactic nature of hay fever.

119 (1701)

The early effects of conjugation on the division rate of Spathidium spathula.

By LORANDE LOSS WOODRUFF and HOPE SPENCER.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Conjugation occurred readily in a pedigree culture of *Spathidium spathula* and therefore experiments were started to determine the effects of fertilization in the life history of the organism. During the first six months of the work, more than sixty lines were derived directly or indirectly from the parent line by conjugation. Some of the exconjugant lines studied represent the F_1, F_2, F_3, and F_4 generations. All the lines which are compared were bred under identical cultural conditions.

A comparison of the number of generations attained by each exconjugant line with that attained by its parent line during the first 15 days after the former's origin gives the following results. Forty-two exconjugant lines produced more generations, eight produced less generations and two produced essentially the same number of generations as their respective parent lines. The various cases in which the parent line did not survive the first fifteen days after the exconjugant line was derived from it are not comprised in these data. If such cases were included it obviously would increase the number of plus cases of exconjugant lines.

Analysis of the data thus far obtained inevitably leads to the conclusion that the exconjugant lines of this pedigree culture of *Spathidium*, under the conditions of the experiment, exhibit, in the great majority of cases, a higher division rate for the first fifteen days after conjugation than the parent lines.

The evidence to date also indicates that exconjugant lines which are derived from old parent lines (*i.e.* from lines which have undergone many generations since conjugation) show a relatively greater increase in the division rate, during the first fifteen days, as compared with the parent lines, than do exconjugant lines which are derived from young parent lines (*i.e.*, from lines which have more recently conjugated).

The complete paper will appear in the *Journal of Experimental Zoölogy.*

120 (1702)

Comparative study of ethanol, caffeine and nicotine on the development of frogs' larvæ.

By **D. I. MACHT** and **WM. BLOOM.**

[*From the Pharmacological Laboratory, Johns Hopkins University.*]

The effects of ethanol, caffeine (alkaloid) and nicotine (alkaloid) solutions were studied on the growth and development of the larvæ of two species of frogs, viz., *Rana sylvatica* and *Rana palustris*. The study of the larvæ was begun immediately after hatching from the eggs and continued on tadpoles of older ages. Tadpoles of the same species and ages were placed in solutions of the above drugs of various concentrations and the effect of the poisons was noted.

It was found that the toxicity of the drugs varied with the age of the tadpoles and with the nature of the drug. Very young tadpoles succumbed much more quickly to all the poisons than the older ones when placed in solutions of the drugs of the same concentration. Tadpoles 1 and 2 days old quickly died in all the solutions unless the drugs were present in a very dilute concentration. Older tadpoles continued to live in solutions of all the drugs longer than was at first expected. The most toxic of the 3 drugs in point of dosage was nicotine. Next in toxicity came caffeine and the weakest of the 3 drugs studied was ethanol. It was surprising to find that while caffeine even in great dilution proved deleterious to the growth and development of the tadpoles, ethanol affected the animals to a much lesser degree. Thus it was found that while tadpoles of the age of 8 days when placed in a solution of nicotine, 1–50,000 succumbed on the 23d day, other tadpoles of the same age placed in caffeine solution, 1–10,000 died on the 12th day, while still other tadpoles of the same age placed in a solution of ethanol, 1–100, by volume, lived as long as 40 days. It was furthermore noted that tadpoles placed in solutions of ethanol, 1–500, lived even longer and appeared to be but slightly affected by the drug. Further experiments on the subject are in progress. This investigation was begun in the spring of 1920.

121 (1703)

The nutritive properties of milk with special reference to growth and reproduction in the white mouse.

By **H. A. MATTILL.**

[*From the Department of Physiology, University of Rochester, Rochester, N. Y.*]

In a recent paper[1] from this laboratory some success was reported in correcting the failure of rats to reproduce by the addition of yeast to a ration of powdered whole milk. The value of yeast had been indicated by some simultaneous experiments on the white mouse. As was suggested by Miss Wheeler some years ago,[2] the mouse, unlike the rat, cannot grow normally on an exclusive diet of dry whole milk. Of the two limiting factors which she indicated, protein and inorganic salts, the latter appears to be the more important; but on a dried milk ration supplying both of these factors in added amounts the rearing of young has not been generally successful. On a food consisting of dried whole milk 93 per cent., salt mixture 2 per cent. and yeast 5 per cent., we have now obtained a fourth generation. Animals on this food without yeast (98 per cent. milk powder, and 2 per cent. salt mixture, with an additional 0.2 per cent. Fe citrate) become pregnant and young are born, but they are small and scrawny in appearance and usually die within 3 or 4 days. Sometimes no trace is found of these litters, the only evidence being the drop in weight of the female. What constituent of yeast is responsible for the successful reproduction secured by its addition remains to be determined by the work at present under way.

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[1] Mattill, H. A. and Conklin, R. E., *J. Biol. Chem.*, 1920, xliv, 137.

[2] Wheeler, Ruth, *J. Exp. Zoöl.*, 1913, xv, 209.

122 (1704)

The presence of vitamine A in the peel of common citrous fruits

By **ETHEL COOPER** (by invitation).

[*From the Hull Physiological Laboratory, The University of Chicago.*]

About a year ago, preparations of orange peel were added to a diet otherwise free from the fat soluble vitamine. The possibility that such preparations might contain fat soluble A was based on the hypothesis of Steenbock[1] that the fat soluble vitamine is a yellow vegetable pigment or a closely related chemical compound.

The peels used for a determination of their fat soluble vitamine content were faultless and of the deepest yellow color. The outer surface of the dried peels was grated enough to break the tiny pockets which contain the yellow oil. Peels and gratings were then extracted on the water bath with ether and alcohol. These extracts were evaporated down to dryness. The gummy mass thus obtained was stirred thoroughly into a diet otherwise free from fat soluble vitamine and then fed to a number of white rats. The results yielded indubitable evidence that the waxes and oil of the orange peel are rich in the fat soluble vitamine. On this diet young rats have matured, mated, and raised young. Control experiments showed the diet, without the preparation of orange peel, to be free from fat soluble A.

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[1] Steenbock, *Science*, 1919, i, 352.

Experiments now in progress indicate that similar preparations made from lemon and grape fruit peel likewise contain the fat soluble vitamine.

A detailed account of the experiments will appear in the course of the year.

123 (1705)

Effects of age and of the inclusion of salts on the heterotropic action of colloidal bodies of cytological interest.

By **D. T. MACDOUGAL.**

[*From the Desert Laboratory, Tucson, Arizona.*]

Auxographic measurements of the swelling of sections of dried plates of agar and of gelatine previously described show that the relative enlargement of a colloidal body in its different axes will be determined largely by the unequal stresses which may be set up, as for example when liquid agar or gelatine is poured on glass and dried without shrinking in area. It was pointed out that sections from such plates of agar increased only 3 or 4 per cent in length while swelling 3,000 or even 4,000 per cent in thickness, and that sections of gelatine increased 8 to 40 per cent in length while swelling from 500 to 2,000 per cent under the auxograph.[1]

Tests of sections of plates of pure agar freshly made and a year old have recently been made. Plates which swelled 2,000 per cent in water when freshly made August 1, 1919, increased but 1,600 per cent July 1, 1920. Plates swelling 3,200 per cent when young increased but 2,000 per cent when nine months old. This total decrease was accompanied by lessened swelling in thickness and increased swelling parallel to the broad surfaces of the plates. The relative increase in length and width of sections of old plates was double that in the same plates when newly made and swelled in water. Similar increases occurred when old plates were hydrated in chlorides of K, Na, Mg and Ca at 0.0001 *M*.

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[1] MacDougal and Spoehr, "Hydration of Amino Compounds," PROC. SOC. EXPER. BIOL. AND MED., 1919, xvii, 33–36.

The effects of age on gelatine plates are not so marked but the areal swelling increases with age. The differential effects of the various solutions on such areal or linear increases were very marked and noticeable. Thus strips 30 to 50 mm. in length cut from a single plate when placed in the solutions gave increases in thickness and length as below:

	.01 M.		0.001 M.		0.0001 M.	
	Th.	L.	Th.	L.	Th.	L.
KCl	1090 p. ct.	38 p. ct.	800 p. ct.	33 p. ct.	640 p. ct.	35 p. ct.
CaCl......	600 "	6 "	1350 "	8 "	830 "	10 "
HCl	1620 "	70 "	1600 "	20 "	925 "	8 "
Water.....					780 "	8 "

The areal increase in the potassium solution varied but little in the different concentrations being much greater than in calcium, which was near that in water. The greatest disproportion however between increase in thickness and in length was in the acid.

Agar was made into plates with an inclusion of minute proportions of chlorides of calcium, potassium and magnesium, which would represent possibilities in the plant cell. When such salted plates were hydrated in solutions of KCl, NaCl and HCl at 0.0001 *M* the swelling in length amounted to 12 to 14 per cent. as compared with increases of 3 to 4 per cent. which might be shown by pure agar.

The increase in length of the sections of salted gelatine cut from plates cast to harden heterotropically are as below:

	0.01	0.001 M.	0.0001 M.
KCl..............	14 per cent.	14 per cent.	12 per cent.
CaCl.............	12 " "	8 " "	12 " "
HCl..............	80 " "	15 " "	8 " "

Among the more important effects it is to be seen that the increase in length of heterotropic plates is lessened by the incorporation of salts when swelled in KCl. The presence of incorporated salts accelerates increases in length in $CaCl_2$ in an uncertain manner, but exercises such an effect rising with the concentration in acid. The presence of incorporated salts lessens the increase in length in KCl, does not modify it greatly in HCl, but exaggerates the increase in $CaCl_2$ at 0.01 *M*.

The alterations in dimensional relations resultant from age, from the incorporation of salts in concentrations within the range of occurrence in the cell, and from hydration in various solutions are to be included in the possibilities of conditions affecting growth and cytological procedure.

124 (1706)

Is glycogen the source of acids developed in autolysis?

By **WITHROW MORSE.**

[*From the School of Medicine, West Virginia University, Morgantown.*]

In the following communication, an attempt is made to answer the question whether glycogen contributes to the rise in acidity in an autolyzing digest.

Method.—Beef liver from the butcher was ground in sand, diluted with Ringer's Solution to make a 20 per cent. digest by weight and divided into two portions, I (control) and II, to which one gram of glycogen obtained from liver was added for every 250 c.c. digest. In order to follow the rate and extent of digestion, the following procedure was used: Fifty c.c. of the well-mixed digest were transferred to a 100 c.c. volumetric flask and made up to the mark with 5 per cent. trichloracetic acid. The mixture was left until precipitation was completed (4 to 12 hrs.) and then filtered. The nitrogen in 20 c.c. of the filtrate was then determined by Sorensen formol-titration. The reaction of medium was studied by the following method: Fifty c.c. of the digest were placed in fish-bladder dialyzing sacs and dialysis was made against Ringer's Solution for 10 hrs. Hydrogen ion concentration was then determined by the gas chain method, a Leeds and Northrup Type "K" potentiometer, Weston standard cell and platinum needle contact electrode being used. For the privilege of using the Government apparatus in the West Virginia Experiment Station, the writer thanks Professors McIlvane and Morgan.

In the following protocol, the averages of triplicate experiments are given. The rate is given in cubic centimeters of decinormal nitrogen, the hydrogen ion concentration in the Sorensen nomenclature (P_H):

	Initial.	24 Hours.	Eleven Days.
I.	3.8 c.c.	5.25 c.c.	9.00 c.c.
P_H =	6.42	7.00	6.04
II.	3.60 c.c.	6.50 c.c.	10.60 c.c.
P_H =	6.60	6.77	5.79

At the end of the period (eleven days), the remaining glycogen, if any, was sought and it was found that 13 milligrams computed as glucose from Benedict determinations on the hydrolyzed (acid) filtrates, remained of the gram introduced, at the beginning.

Discussion.—It is evident from the results of the experiment that we may look to glycogen as one of the precursors of substances concerned with the development of acidity in autolyzing tissues. These substances are probably hydroxy acids, such as lactic and keto-acids, such as pyruvic. Obviously, it is possible for such acids to form from other sources, as for instance, from the carbohydrate moiety of nucleosides, from the deaminized residue of amino-acids and doubtless neutral fats and phospholipines likewise may contribute. The interest in glycogen for the writer centers about the increased metabolism at the inception of starvation. A well-fed guinea pig will exhibit a sudden increase in amino-nitrogen when its carbohydrate food is limited or replaced by nitrogenous diet. The disappearance of glycogen from the liver is remarkably fast. Thus a guinea pig whose diet had been controlled from February 16 to February 23, having been given a full carbohydrate diet during this period was permitted to starve three days and an examination of the liver showed the complete absence of glycogen. The fat of the pig does not seem to change correspondingly with the change in diet and the suggestion is made that the rapid rise in nitrogenous excretion, expecially of amino-nitrogen may be due to the contribution of glycogen to the increase in hydrogen ion concentration with the concomitant appearance of the optimum reaction for tissue enzyme action which Dernby has shown to occur. Intra vitam autolysis then proceeds.

Aside from the reports of Bradley, Dernby and of the present writer, this phase of the dynamics of tissue enzyme action does not seem to have been examined.

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MORSE, W. *J. Biol. Chem.*, 1917, xxx, 197.

125 (1707)

Inoculation of alastrim or West Indian smallpox.

By **J. P. LEAKE** and **J. N. FORCE**.

[*From the Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.*]

For about twenty years there has been observed in tropical and subtropical America an eruptive disease of very low mortality, the identity of which with smallpox has been a subject of question, especially since previous epidemics of smallpox in these regions have been attended with high mortality. This disease outside the United States has been variously termed alastrim, varioloid varicella, and kaffir pox. It has been asserted that a point of difference between this disease and true smallpox was the resistance of lower animals to inoculation; no positive result from inoculation with West Indian or South American strains has been reported in any of the available literature, though Aragao[1] described the development of Guarnieri bodies in the cornea of rabbits inoculated with this disease.

Through Professor W. G. MacCallum, of Johns Hopkins Medical School, pustule contents preserved in 0.5 per cent. phenol at a low temperature for several months, were obtained from two Jamaican cases; also through Lt. Com. G. F. Clark, U. S. N., crusts, preserved dry for two weeks, were obtained from a case in Haiti. These were used for the cutaneous inoculation of two *Macacus rhesus*, which showed no reaction other than a serous exudate at the site of inoculation for eight days, when an eruption developed at two of the three sites of inoculation on each monkey. The second of the two Jamaican viruses gave no result on either monkey. The typical lesion consisted of a papule with reddened periphery surrounding a white area with a brownish depressed center. The lesions were discrete, five to eight in number in three of the sites inoculated, and confluent in the fourth. No complete vesiculation appeared, but the itching was evidently severe, since the monkeys abracted the tops of the lesions. These were at their height on the eleventh day, when they showed marked elevation, reddened base, crater formation, and serous exudation. Curettings from one of the monkeys, at this stage, produced no results when inoculated cutaneously on three rabbits, two of which had proved refractory to similar inoculation with the original alastrim material from human cases. The monkeys had no generalized eruption or other symptoms.

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[1] Aragao, "A proposito do Alastrim," *Brazil medico*, March 15, 1911. Rev. in *Bul'. Inst. Past.*, 1911, ix, 942.

Thirteen days after the monkeys were first inoculated with alastrim, one of them was reinoculated with one of the two strains (Jamaican) which had given a successful result, and the other, as well as a control monkey, was vaccinated with an active vaccine virus. The control monkey developed a typical primary vaccinia beginning on the fourth day and reaching its fastigium on the ninth day. The monkeys previously inoculated with alastrim showed nothing beyond a transient reaction of immunity, or of trauma, at the sites of inoculation with vaccine virus and of the second application of alastrim material, respectively.

This immunity to vaccinia is at least as high as that produced by the American strain of smallpox. Three monkeys, some months previous to the above series, were inoculated with vesicle contents from smallpox and developed local lesions similar to those above described. Twenty-four days later they were vaccinated, together with three controls, and showed a definite vaccinal "take," though more rapid and less severe than the previously uninoculated monkeys, *i.e.*, a vaccinoid or accelerated reaction.

126 (1708)

Studies on the action of mercury.

By **WILLIAM SALANT** and **NATHANIEL KLEITMAN**.

[*From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

Observations on the pharmacology of mercury were made, with special reference to its influence on the circulation and respiration. Mercury in a concentration of 1:5000, in the form of the benzoate, succinate and acetate, was injected intravenously into dogs and cats under ether or ether-paraldehyde anesthesia.

Small doses of mercury frequently produced pronounced changes in the circulation as well as respiration. One and a half to two milligrams per kilo caused stimulation of respiration. Frequency and particularly depth of respiration were increased shortly after the administration of the salt was begun and the effect sometimes persisted for several minutes after the injection was discontinued. Larger doses, however, produced the opposite effect. Respiration was also depressed when the injection of a stimulating dose was repeated several times, thus indicating cumulation.

The effect on the circulation was more complicated. Small doses usually produced a temporary rise in blood pressure of about 8–10 mm. Hg, but in several experiments no change was observed. When small doses were repeated so that the total amount injected was 4–5 mg. of mercury per kilo, depression of the circulation was observed, thus showing cumulation as in the case of respiration. The changes produced usually consisted of a sudden drop in blood pressure and slowing or arrest of the heart which lasted in some experiments thirty seconds. This was followed by recovery, the blood pressure rising rapidly and attaining even a greater height than that before the injection. Very often the blood pressure remained at the new high level for some time and then descended again, but the descent was gradual. The sudden fall in blood pressure was a frequent occurrence, however, in a number of experiments, and in a few cases no recovery took place.

Attention may also be called in this connection to the long latent period which very often preceded the sudden fall in blood pressure, two to six minutes elapsing before this occurred.

The effects on blood pressure were different when the vagi were cut before the administration of mercury was begun. The lowering of blood pressure was much less abrupt and longer in duration. In one experiment it lasted for more than fifteen minutes. Marked changes in heart action were also observed. It might be added that stimulation of the peripheral end of the vagus failed to elicit the usual response after a sufficient amount of mercury was injected.

127 (1709)

The toxicity of skatol.

By **WILLIAM SALANT** and **NATHANIEL KLEITMAN.**

[*From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

Experiments with skatol (Kahlbaum, highest purity) were performed on frogs, cats and dogs. 30–40 mg. of skatol dissolved in 0.3–0.4 c.c. of pure acetone and injected into the ventral lymph sac of frogs weighing 40–45 grams produced symptoms of severe intoxication within a few minutes. Respiratory movements became slow, reflexes gradually disappeared, muscular weakness was followed by complete paralysis. Death occurred in from thirty minutes to three hours. In control experiments with the same or larger amounts of acetone (0.5 c.c.) similar symptoms were observed, but they were much less pronounced and were followed by recovery. When the frogs were examined after 18–20 hours they appeared perfectly normal.

When injected intravenously into cats and dogs, skatol produced a marked and persistent fall in blood pressure, with slow recovery. As in the case of the frogs, these experiments were controlled by injecting equal or larger amounts of acetone, the speed of injection being the same, but the fall in blood pressure produced was not so pronounced and was followed by immediate recovery. In one experiment on a dog (6 kilos) which received 50 mg. skatol in one c.c. acetone intravenously blood pressure fell promptly from 165 to 90 mm. Hg, the recovery occupying seven minutes. One c.c. of acetone injected with the same speed caused a fall in blood pressure from 160 to 120 mm. Hg, and was followed by prompt recovery. In another experiment on a cat (2.2kilos) blood pressure fell thirty-six per cent. after the injection of 30 mg. skatol in one c.c. of fifty per cent. acetone. Four minutes after the injection recovery was still incomplete. Two c.c. of fifty per cent. acetone alone, when injected intravenously into the same cat, produced a fall in blood pressure of only sixteen per cent., which was followed by an immediate rise exceeding the original blood pressure.

128 (1710)

The concentration of sodium and potassium as compared with that of calcium and magnesium in the serum of patients with active infantile tetany.

By **F. F. TISDALL, B. KRAMER** and **J. HOWLAND.**

[*From the Department of Pediatrics, Johns Hopkins University.*]

We have determined the sodium, potassium, calcium and magnesium content of the serum of children suffering from active infantile tetany. As most of the infants were quite small it was only possible in a few cases to do the four determinations on the serum of the same individual. The results are given in Table I. It is seen that the sodium content falls within the limits of normal. The potassium content is apparently somewhat elevated. The concentration of calcium in the serum, as previously shown, is markedly diminished while that of magnesium is usually within normal limits.

	Sodium Mg. per 100 C.c.	Potassium Mg. per 100 C.c.	Calcium Mg. per 100 C.c.	Magnesium Mg. per 100 C.c.
1.........	324	25.0	5.6	2.2
2.........	330	26.7	6.2	1.6
3.........	337	19.0	6.6	2.1
4.........	323	24.8	5.8	1.7
5.........	322	28.4	5.0	2.9
6.........	324	26.0	5.2	2.4
7.........			3.5	
8.........			5.8	
9.........			6.3	
10.........			6.7	
11.........			6.0	
12.........			6.7	
Average....	327	24.9	5.8	2.1

The average concentration of these elements in the sera of normal children is singularly constant. The actual figures are as follows:

Calcium........................	10 – 11	mg. per 100 c.c. of serum.
Magnesium......................	2 – 3	mg. per 100 c.c. of serum.
Sodium..........................	325 –345	mg. per 100 c.c. of serum.
Potassium......................	18.5– 20.5	mg. per 100 c.c. of serum.

The (Na + K)/(Ca + Mg) ratio in the normal infant is therefore

$$\frac{340 + 19.5}{10.5 + 2.5} = 27.6,$$

while in cases of active infantile tetany it is

$$\frac{327 + 24.9}{5.8 + 2.1} = 44.5.$$

If the calcium were to remain the same, the ratio would be 27.8. It is therefore evident that the change in the ratio of (Na + K)/(Ca + Mg) is due almost wholly to the decrease in the concentration of calcium.

129 (1711)

Serumtherapy of advanced botulism.

By J. BRONFENBRENNER and H. WEISS.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston.*]

In the course of a series of investigations designed for the purpose of establishing the path of absorption of botulinus toxin in guinea pigs, a number of animals were kept under ether for the purpose of surgical manipulation. It was observed that in such animals death following the introduction of toxin was greatly delayed. Whereas normal guinea pigs of 350 grams given 50,000 minimal lethal doses[1] of botulinus toxin intraperitoneally show symptoms of dyspnea in one hour and invariably die in about two hours, guinea pigs similarly injected but put under ether anesthesia for two hours as soon as dyspnea occurs (*i.e.*, one hour after the injection of toxin) will survive for four hours and by prolonging the period of anesthesia, the life of the animals can be correspondingly prolonged.

We thought that advantage could be taken of this delay in the rate of the progress of the intoxication under ether anesthesia to permit toxin antitoxin combination to take place. Two series of guinea pigs were given 50,000 minimal lethal doses of botulinus toxin per os. Guinea pigs thus fed show first symptoms of intoxication in about six hours and die in about twelve hours. After six hours the first series received antitoxin intravenously while the second series was given antitoxin in a similar manner but at the same time put under ether anesthesia which was continued for two hours. The pigs in the first series died in eighteen hours while those in the second series survived.

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[1] The minimal lethal dose used throughout this paper is the amount that is necessary to kill a mouse of 15 grams in less than four days by intraperitoneal injection.

Further experiments are being carried out which attempt to gain an insight into the nature of the phenomenon, how far it can be applied with relation to other toxins and the effect of other anesthetics. The quantitative and time relationships are also being studied.

130 (1712)

The composite nature of botulinus toxin.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston.*]

As we will show in detail in another paper, the lethal dose of botulinus toxin by the mouth is roughly 1,000 removed from that sufficient to kill by the intraperitoneal route. This relation seems to hold true for all the laboratory animals which we investigated, including birds, and is responsible for the failure of certain investigators to kill birds by feeding even large quantities of weak toxin.

In attempting to purify the toxin by precipitation, we were surprised to find that, whereas the purified toxin retained its full potency when tested by injection, it became 100 times less toxic by mouth. In general the further the purification was carried, the greater was the loss in potency of purified toxin when tested by mouth. We have been able to reëstablish the toxicity (by mouth) of such purified toxin by merely adding to it the substances removed by the process of purification.

Since the potency of our purified botulinus toxin as tested by injection remains the same, whereas the toxicity by mouth varies according to the degree of purification of the toxin, it seems to us that the power of crude botulinus toxin to be absorbed through the intestine is dependent upon the presence of secondary substances mixed with the true botulinus toxin.

131 (1713)

Superinoculation experiments with Treponema pallidum.

By **WADE H. BROWN** and **LOUISE PEARCE.**

[*From the Rockefeller Institute, New York City.*]

The majority of investigators have interpreted the results of superinoculation experiments with *Treponema pallidum* as showing that one infection affords protection against another. The chief criterion for determining results has been the production of a characteristic lesion containing spirochetes, it being virtually assumed that if no lesion occurred no infection had taken place. When it is recalled that the lesion produced at the portal of entry in a first infection may be very slight or entirely absent[1] and that organisms may multiply in the body for months or even years without giving rise to any external manifestation of disease, it is obvious that such a standard of measurement is of more value as an index of the ability to produce a manifestation of disease than of infection, and that infection cannot be excluded upon this basis. It would appear, therefore, that before the results of superinoculation experiments can be made clear, the subject must be approached from a broader point of view and that evidence must be adduced which will enable one to see beyond the reaction at the site of inoculation.

With this idea in view, a large series of superinoculation experiments was carried out on rabbits with five strains of *Treponema pallidum* representing organisms of a wide range of virulence for these animals. These strains included the highly virulent ones of Nichols and of Zinsser and Hopkins, isolated in 1912 and 1913, and three less virulent strains, isolated during the fall of 1919. In general, the animals were first inoculated in one or both testicles while the second inoculation was made intracutaneously on the sheath or at the base of one ear using equivalent doses of a testicular emulsion. The reaction to superinoculation was studied from the period of early primary infection to that of latency following spontaneous healing of primary or generalized lesions including both the local reaction and the subsequent course of the disease. Especial attention was given to conditions which appeared to influence either of these reactions and the results were controlled by parallel observations on the reaction produced by the infection of normal animals.

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[1] Brown, W. H., and Pearce, L., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 200.

While all of the details of the experiments are not as yet available, certain definite results may be recorded in so far as the local reaction is concerned. Briefly, four general types of reaction were observed:

1. The first reaction consisted of a mild, acute inflammatory process which was followed by a papular infiltration in the skin associated with more or less enlargement and induration of the regional lymph nodes. In its typical form, this reaction began within 24 hours and reached its height within 5 to 7 days after inoculation; it then subsided very rapidly, disappearing completely within 9 to 14 days after inoculation.

2. A second form of reaction differed from that described above in only one respect. With the fading of the cutaneous lesions, there was only a partial resolution of the lymphadenitis followed almost immediately by a progressive enlargement and induration which resembled the satellite adenitis of a primary infection in all essential respects. This condition persisted for weeks or even months.

3. With a third group of animals, an additional feature was introduced in the form of a diffuse or papular infiltration of the skin at the site of inoculation. These lesions presented the appearance of early primary infiltrations or of the slight cutaneous lesions frequently observed in primary infections and contained actively motile spirochetes in abundance. The reaction was usually manifest within 7 to 14 days; while the regional lymph nodes exhibited a progressive and persistent enlargement and induration, the cutaneous lesion was transient and usually disappeared within 2 to 3 weeks.

4. The fourth type of reaction was characterized by the development of persistent cutaneous lesions associated with a pronounced lymphadenitis. The cutaneous lesions assumed the form of flattened or elevated papules varying from a few millimeters to a centimeter in cross diameter or developed into large, well

indurated lesions which were difficult to distinguish from primary lesions produced by this mode of inoculation. The differences noted were a more rapid growth and a tendency to early and widespread necrosis with central softening—reactions which in the rabbit are indicative of a malignant turn of the infection.

The gradations in the reactions described (omitting Type 1) may all be observed with a first infection in any considerable series of animals inoculated with well adapted strains of *Treponema pallidum* and the more characteristic ones furnish conclusive evidence that a local infection together with the usual manifestations of disease may be produced by the reinoculation of animals already infected. Further than this, it is by no means certain that infection may not occur also in instances where no lesion is produced at the site of inoculation since the immediate production of a lesion cannot be regarded as an essential criterion of infection.

The character of the results obtained could be definitely related to a number of factors, among which may be mentioned the relative virulence of the organisms used for the primary and for the secondary inoculation, the progress of the original infection (acquired resistance), the presence of actively developing lesions (inhibition), and individual animal variation (native resistance). Finally, reactions of Type 4 which include all of the essential features of the local reaction to a primary infection were obtained with ease in certain animals by the superinoculation of the two older strains of *Treponema pallidum* upon the more recently isolated ones, even after the original lesions had practically resolved. It is thus clear that in given instances, the resistance acquired as a result of infection with an organism of low virulence may never reach the point of an effectual protection against one of high virulence. However, the crucial test of the possibilities presented by the problem of superinfection rests more upon the demonstration of a definite influence upon the course of the disease than upon any form of local reaction which may be produced. This phase of the subject is reserved for a separate communication.

132 (1714)

Multiple infections with Treponema pallidum in the rabbit.

By **LOUISE PEARCE** and **WADE H. BROWN.**

[*From the Rockefeller Institute, New York City.*]

Once it has been demonstrated that under appropriate conditions superinoculation of a rabbit with an advanced syphilitic infection may give rise to a typical primary lesion,[1] the question naturally arises as to whether this second infection is limited in its effects to the local reaction or is capable of further participation in the disease produced.

The problem was approached by a number of experiments. Rabbits infected with strains of low virulence were reinoculated with strains of high virulence after the original infection had become well established. In general, the primary inoculation was made in one or both testicles while the second was intracutaneous on the sheath or at the base of one ear, using equivalent amounts of a testicular emulsion. The infections thus produced were compared with those in a series of control animals. The interpretation of the experimental results was based upon the usual course of the disease produced by each strain with particular reference to the type and severity of lesions and to their time and sequence of occurrence since at a given time and under given conditions these are comparatively constant properties of any given strain.

The experiments up to the present time have yielded a number of instances in which the nature of the infection differs from that ordinarily seen with any one of the several strains employed and this may be illustrated by citing a single example. The two strains used in this experiment have been studied in a large series of animals. The less virulent strain (III) used for the primary inoculation was isolated in the fall of 1919. It has always produced a mild infection with slight primary lesions of short duration and generalized lesions of a minor character consisting of occasional small diffuse or papular lesions of the skin, slight infiltrations about the sheath, a few cases of keratitis and two instances of slight periostitis of the nasal bones. The infection produced by the Nichols strain, which was isolated in 1912, has been much more severe with pronounced primary lesions and the frequent occurrence of extensive lesions of the bones, large cutaneous granulomata, lesions of the mucous membranes and of the eyes. The time of occurrence of generalized lesions with both strains is subject to wide variations but in general, is from 2 to 3 months after inoculation. In the vast majority of instances, lesions of the periosteum and bone, skin, and eyes appear in the order given.

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[1] Brown, W. H., and Pearce, L., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 200.

The rabbit was inoculated into the right testicle with Strain III on December 2, 1920, with the production of an orchitis, which pursued a normal course. On January 26, 1921, 55 days after inoculation, the rabbit was reinoculated in the sheath with the Nichols strain; at this time, resolution of the testicular lesions was well advanced and the inguinal lymph nodes were barely palpable.

During the following week, a definite papule developed on the sheath; the inguinal lymph nodes became enlarged and indurated and activity of the orchitis was resumed. 19 days after reinoculation, the lesion on the sheath was much larger than in any of the controls and dark field examination showed actively motile spirochetes. In addition, marked metastatic lesions were present in the left testicle.

3 weeks after reinoculation, or 2½ months after the primary inoculation, there was a large actively growing chancre, an intense metastatic orchitis, marked popliteal adenitis, a keratitis of the right eye, and a number of bone lesions which resulted in extensive necrosis of the nasal and tarsal bones and slight necrosis of the distal end of the right ulna. The animal also showed pronounced emaciation and weakness. During the next few weeks, the keratitis completely disappeared and has not recurred and the nasal lesions were also healed. The destruction of the tarsal bones, however, was more extensive than has been observed in any other animal infected with any strain of *Treponema pallidum* and during the 2 months since the appearance of the lesions, very little repair has been accomplished, a condition never before observed. The chancre showed irregular periods of growth and

resolution with a pronounced tendency to necrosis and central softening.

This was the condition of the animal 2 months after reinoculation at which time a second group of lesions developed beginning with a reinduration of the chancre and an increase of the lesions in the left testicle. These were followed by the appearance of a granuloma of the left tendo-Achillis and of large cutaneous granulomata on the tail, on the left hind foot and a little later in both scrota. All of these lesions are still present.

The outstanding features of the infection in this animal are the sequence of events and the type and destructiveness of the lesions. The first unusual feature was the remarkably rapid development of the chancre at approximately the same time with a keratitis and extensive bone lesions. Obviously, the occurrence of the chancre can be attributed to the reinoculation. If the keratitis is also referred to the Nichols strain, its time of appearance, 3 weeks after reinoculation, is the first instance of this kind we have so far observed. On the other hand, if it is attributed to the original inoculation, its occurrence 2½ months later, is more in accord with our experience of the time relation of generalized lesions. The subsequent development of a number of prominent cutaneous and tendon sheath lesions some weeks after the keratitis, is another peculiar feature of this experiment, since eye manifestations are usually terminal events of an infection. The only bone lesions observed with the strain used for the primary inoculation have been two instances of slight periosteal lesions of the nasal bones and while destructive bone lesions are comparatively common with the Nichols strain of *Treponema pallidum*, they have never reached the proportions of those observed in this instance. In point of time, moreover, the occurrence of such bone lesions 3 weeks after inoculation is unprecedented in our experience.

The large cutaneous lesions which appeared some 2 months after reinoculation are of the usual type observed with the Nichols strain but differed markedly from the comparatively rare cutaneous lesions of Strain III, which are of the papular or infiltrative type. Moreover, if these granulomata were due to the original inoculation, they should have occurred some time before the keratitis. In their type, time of occurrence and sequence relationship, therefore, these lesions are more closely related to the second than to the first inoculation.

Viewed in this light, it would be difficult to attribute the infection produced in this animal to either of the strains of *Treponema pallidum* with which it was inoculated and although the outstanding manifestations of disease conformed more to the general character of the infection produced by the second than by the first organism, it would seem to be more logical to regard the infection as one produced by the combined action of the two organisms. These results are presented as a means of directing attention to the possibilities of double or multiple infection where appropriate conditions exist rather than to the probabilities of their occurrence.

133 (1715)

Report on anaphylactic deaths in guinea pigs from intracutaneous injection of small amounts of egg albumin.

By **HANS ZINSSER** and **S. T. WU**.

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

In view of the importance of the many problems arising in regard to the relationship between intracutaneous skin reactions and true anaphylaxis, the following laboratory "accident" would seem well worth reporting. It seems especially interesting in connection with the account of a human case of anaphylaxis following the intradermal injection of egg protein which was published by Goestenberger and Davis, and which bears out certain views concerning the relationship of bronchial musculature and anaphylaxis brought out by Wells[1] in his recent critical articles in the *Physiological Reviews*. The occurrence which is unique in our experience with anaphylactic guinea pigs, was as follows:

Six guinea pigs, 341, 349, 62, 568, 1122, and 1119, three males and three females ranging in weight from 235 grams to 265 grams, were treated on February 9 with intraperitoneal injections of 2 c.c. of a solution of crystallized egg albumin, representing about

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[1] Wells, *Physiological Reviews*, 1921, i, 44.

6.6 per cent. dry weight. The guinea pigs were used in experiments in which parallelism between protein skin reactions in hypersensitive animals and similar skin reactions carried out in tuberculin sensitive animals, was being investigated.

On February 12, 0.1 c.c. of a 1–4 dilution of 6.6 per cent. albumin solution was injected intracutaneously into guinea pig No. 349. No reaction resulted.

On February 15, 0.1 c.c. of a 1–4 dilution of the original 6.6 per cent. egg albumin solution was injected intradermally in all the pigs with three additional controls. The reactions were watched from the time of injection for two hours, at very frequent intervals, and after that, at longer intervals. At the end of twenty hours, No. 1122 showed what was considered a moderate reaction, that is, an area of erythema, about one centimeter in diameter, slightly elevated and slightly edematous. In No. 568 and No. 1119 there was slight erythema, at the site of injection, regarded as probably negative since similar erythema was present in controls.

On February 18, a second skin test was done on all the pigs with 0.1 c.c. of crystallized egg albumin solution representing about 11 per cent. estimated by dry weight, from a distilled water solution. The injections at this time were carefully made by the two writers together, and great care was taken to place the entire amount intracutaneously, well-defined white wheals being formed during the injection. Since no adverse symptoms were expected immediately, all the pigs were put into a wire basket and carried into the next room. Within a few minutes, No. 1122, No. 349 and No. 1119 became sick, and No. 1122 and No. 349 were dead within three minutes. It was unfortunate that the actual condition of these pigs during these three minutes was not more carefully observed, since they were dead by the time the basket was again picked up, which was just three minutes after injection. From the condition of No. 1119, however, the symptoms can be inferred, since this pig showed symptoms after three minutes, with a tendency to fall down and very labored respirations, which continued for about an hour, gradually improving during this time. This pig survived. Immediate autopsies done on the two other pigs which died showed typical inflation of the lungs, the heart still beating in the first one autopsied. In every way these pigs resembled pigs dead of anaphylaxis. The wheal at the point of inoculation had not materially changed in size, the inference being that the 0.1 c.c. of the 11 per cent. egg albumin had been absorbed in part only.

No comment is made on this occurrence, and it is reported only as a very peculiar accidental observation which is of great importance to us in connection with the general question of sudden anaphylactic death from minute doses of antigen injected intracutaneously. It appears to be an example of acute death in individual sensitized guinea pigs by the absorption from the skin of very minute amounts of a reasonably pure protein. Attempts to repeat this result with a dozen guinea pigs, since that time, have not succeeded.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One hundred sixteenth meeting.

Columbia University, May 20, 1921.
President Wallace in the chair.

134 (1716)

Variations in the streptolysin curve in serum media.

By **FRANKLIN A. STEVENS** and **CLIFFORD L. LAMAR.**

[*From the Medical Clinic of the Presbyterian Hospital, Columbia University, New York City.*]

In the growth of hemolytic streptococci in serum media, the hemolysin, titrated from hour to hour, shows a gradual ascending curve of concentration which reaches a maximum at about the sixth to the twelfth hour of growth, and then falls rapidly. This curve may be modified by the character of the culture used to seed the media, by the quantity of serum present, the source of the sera, and the amount of peptone in the broth. There are two variations which occur; variations in concentration of hemolysin, and in the time that hemolysin first appears in the culture and later the time at which the maximum amount can be demonstrated. With similar seeding, and only one variant introduced in experiments, these variations are brought out very sharply. Cultures in 20 per cent. rabbit serum and horse serum in broth without peptone show that rabbit serum media allows the production of the lysin sooner and does not give as great concentrations as horse serum. The lower percentages of these sera, when seeded with different plain broth cultures give greater variations in the maximum titre, but these variations are largely obviated in 20 per cent.

media. Twenty per cent. horse serum gives practically constant titres at some time during the growth. The time relationships hold in all concentrations. Peptone (2 per cent.) slightly increases the titre and gives identical titres in 20 per cent. horse serum, and brings the variations within closer limits in rabbit serum media. The time relationships are directly dependent on the growth of the streptococci, so that the delay in lysin production in horse serum indicates a lag in the rate of multiplication during the early hours after seeding. Variations in the nature of the seeding can be obviated by seeding from a 20 per cent. horse serum broth in 2 per cent. peptone, in which all streptococci of human origin, whether virulent or avirulent, reach the same condition in regard to growth and number at the end of 16 hours, unless agglutination occurs.

135 (1717)

The failure of rats to develop rickets on a diet deficient in Vitamine A.

By **A. F. HESS, G. F. McCANN** and **A. M. PAPPENHEIMER.**

[*From the Department of Pathology, College of Physicians and Surgeons, New York.*]

The fat-soluble vitamine, or a vitamine closely associated with it, is regarded by some as being equivalent to an antirachitic vitamine; the development or non-development of rickets is thought to be dependent mainly on the absence or presence of this factor. In a previous report one of the writers has stated it to be his opinion that this relationship does not hold true for human rickets. The following experiments were planned to ascertain whether a diet markedly deficient in this vitamine led to the development of rachitic lesions in young rats; for this purpose rats weighing about 30 g. were put on a diet of extracted casein 21 per cent., rice starch 57 per cent., salt mixture 5 per cent., Crisco 17 per cent., yeast extract (Osborne and Wakeman) 60 mg. a day. The casein was extracted by means of ether and cold alcohol. The salt mixture was that employed by Osborne and Mendel. Thirty-five rats in all were used for this experiment. They were kept on this rigid diet for a period averaging about three to four months, but extending in several cases five to six months. In no instance were rachitic lesions noted either microscopically or macroscopically, merely an inactive osteogenesis. That the dietary in point of fact did contain only a minimal amount of the fat-soluble vitamine was proved by the lack of gain of the animals after they had been on this food mixture for about sixty days, by their prompt response in growth on the addition of 6 per cent. of butter fat to the diet, and by the development of ophthalmia or keratomalacia in almost all of the animals and its rapid subsidence on adding butter to the dietary. In addition to eye lesions the rats on a restricted diet developed many infections. Those receiving 0.5 c.c. of orange juice did not, however, develop either ophthalmia or infections as frequently as others where the diet did not include this food. Ten control animals which received the same diet, with, however, an addition of butter, did not develop ophthalmia, grew normally and remained in perfect health. Our deduction from these experiments is that a lack of the fat-soluble vitamine in a dietary which is otherwise complete does not lead to the development of rachitic lesions in rats.

136 (1718)

The effect of various modifications of a diet producing rickets in rats.

By **A. M. PAPPENHEIMER, G. F. McCANN, T. F. ZUCKER,** and **A. F. HESS.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*]

At the March meeting of this Society, it was shown by Sherman and by one of the writers of this paper that rickets regularly developed in rats maintained on a diet composed of patent flour, calcium lactate, sodium chloride and ferric citrate. It was further found that the substitution of 0.4 per cent. basic potassium phosphate for an equal percentage of calcium lactate in this diet (No. 84), uniformly protected against the development of rickets.

These experiments have been continued. The diet has been modified in various ways, and we wish this evening to report very briefly some of the results obtained.

The basic rickets-producing diet has been tested on a further series of rats, amongst them controls for other experiments. Including the 15 rats referred to in the previous paper, 36 rats in all have been observed to develop rickets upon Diet 84. A few of these after having shown unmistakable rachitic changes by x-ray, were subsequently given other diets. In all the other rats, the diagnosis has been confirmed by microscopic examination. It may be confidently stated then that rickets will develop in 100 per cent. of animals upon this diet; at least, in our experience, there have been no exceptions.

The protective action of the basic potassium phosphate has also been demonstrated in 9 additional rats; and there have been no failures amongst the total 24 rats, which have been studied up to date.

The first question to be answered was as to the part played by the potassium and the phosphate respectively, in the protection afforded by the basic potassium phosphate. To determine this point, an equivalent amount of primary *sodium* phosphate was substituted for the potassium salt in the diet; and in another series of the same litter, an equivalent amount of potassium *chloride* for the potassium phosphate. The results of this experiment are shown in Table II, from which it is apparent that the protection is conferred by the *phosphate*, and not by the *potassium.*

Experiments to determine the minimal amount of phosphate (calculated as P), which, when added to the basic diet 84 will afford complete protection, are not yet completed. The data given, however, indicate that this limit lies between 50 and 25 mg. per cent., the original diet 85, containing 72 mg. of added phosphorus per 100 gms. of diet, in addition to the 87 mg. contained in the basic diet 84.

As was pointed out in the previous paper, Diet 84 is inadequate for proper growth, being deficient not only as to the character of the protein, but in both fat soluble A, and possibly in its water soluble B vitamines. The experiments summarized in Table IV show the effect of the addition of various substances to the rickets-producing diet.

1. Four rats receiving an addition of 0.2 gm. of pasteurized butter daily, developed rickets, the lesions being quite as severe as those found in the control rats of the same litter maintained on Diet 84 without butter. Further experiments are in progress, using fresh *unpasteurized* butter. However, there is good reason to believe that the butter used was adequate as regards its content of fat soluble A. Ophthalmia did not occur in the butter-fed rats, but was found in approximately 90 per cent. of the rats on Diet 84 alone. Furthermore, a typical gain in weight was obtained when this pasteurized butter was added to a fat-soluble vitamine deficient diet.

This experiment, we believe, adds further confirmation to the data presented by Hess and his co-workers. In rats, absence of fat soluble vitamine in the diet does not produce rickets, nor does its presence prevent it.

2. The addition of 60 mg. of "Harris yeast vitamine" daily to diet 84 gave complete protection in 3 rats. The phosphorus content of this yeast preparation is so high that it comes within the range which, in the form of an equivalent amount of phosphate, would confer protection. Nothing definite can, therefore, be deduced from this experiment, as to the possible protection attributable to the water-soluble vitamine factor. It is interesting to note that all three rats of this series showed ophthalmia.

3. The addition of 10 gms. of purified casein to 100 gms. of Diet 84 (containing phosphorus equivalent to the 72 mg. present in Diet 85), gave results which are difficult to interpret. All three rats of this series showed in the x-rays taken after 22 days on the diet, distinct rachitic lesions. One of the rats, sacrificed on this day, had definite rickets, grossly and microscopically. The other two were allowed to continue to the 38th day, the x-rays taken at death, showing an apparent healing of the rachitic lesions in the head of the tibia. The microscopic study of the ribs in these rats shows no active rickets. Further experiments are planned to compare quantitatively the protection given by casein with that given by inorganic phosphate.

4. The addition of both casein and yeast greatly improved the growth and nutrition of the rats. As was to be expected from the results with the addition of yeast alone, there was complete protection afforded.

The experiments here reported must be regarded as preliminary to more detailed studies. The ease and certainty with which the disease can be produced in rats cannot but make its experimental study profitable, although it would be obviously premature to apply the data already obtained to the problem of human rickets.

137 (1719)

Diffusible calcium in normal, rachitic, and experimental tetany blood.

By L. VON MEYSENBUG.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*]

In 1911, Rona and Takahashi[1] reported their work on the diffusible Ca of horse, ox, and pig serum, finding an average of 65 per cent. of the total serum Ca to be diffusible. No work was done with human blood. MacCallum, Lambert and Vogel[2] in 1914 made the following statement: "If tetany blood be dialyzed under exactly the same conditions as normal blood, it still loses a proportionate amount of its Ca, which would perhaps show that it is not especially the loss of a diffusible Ca as contrasted with a non-diffusible form—which is important in producing tetany." Brinkman[3] in 1919 advanced the hypothesis that the calcium-ion concentration is dependent on the CO_2 tension of the blood. In view of the altered blood CO_2 combining power found in tetany by some workers, we have endeavored to correlate Brinkman's hypothesis with the low Ca content of the blood in tetany. During the past year, we have worked with human blood and experimental tetany dog blood.

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[1] Rona and Takahashi, *Biochem. Ztschr.*, 1911, xxxi, 336.

[2] MacCallum, Lambert and Vogel, *Jour. Exp. Med.*, 1914, xx, 149.

[3] Brinkman, *Biochem. Ztschr.*, 1919, xlv, 101.

The method of dialysis is an important factor in the results obtained. In the first place, collodion sacs are unsatisfactory, because they do not hold back protein for a sufficient length of time and because there is a progressive passage of fluid into the sac. We have used parchment paper thimbles, which obviate these objections. It was found that when serum was dialyzed against a Ca-free Ringer solution, there appeared to be a progressive dissociation of Ca, so that, at the end of 5–7 days' dialysis, 90 per cent. of the total Ca had diffused out. Where, however, compensation dialysis is employed, *i.e.*, known amounts of Ca are added to the dialyzing fluid outside the sac, equilibrium is obtained in 24 hours, at ice box temperature, and the diffusible Ca is found to be between 60–70 per cent. In all of our work, the serum and dialyzing fluid were saturated with 6 per cent. CO_2-air mixture, and the buffer of the dialyzing fluid was such that when so saturated, it had a p_H of 7.4.

The calculation of the diffusible Ca is simple and may be expressed in this formula:

$$\text{Diffusible Ca} = \frac{(\text{Ca in dialysate} \times 2) - \text{Ca added to dialysate}}{\text{Serum Ca}}$$

In the two tables our results are summarized.

The calcium determinations were done by Lyman's method.

DUPLICATE ANALYSES—EXPERIMENTAL TETANY, DOG NO. 6.

Series NN.	Ca in Serum at Start, mg. in 4 c.c.	Ca in Serum at End, mg. in 4 c.c.	Ca in Dialysate at Start, mg. in 4 c.c.	Ca in Dialysate at End, mg. in 4 c.c.	Serum Ca, mg. in 100 c.c.	Total Ca in System.			Per Cent. Dialyzable Ca. %
						Before mg. in 8 c.c.	After mg. in 8 c.c.	Diff. mg.	
NN 1	0.248	0.274	0.210	0.184	6.1	0.458	0.458	0	60
NN 2	0.248	0.274	0.210	0.180	"	0.458	0.454	0.004	63
NN 3	0.248	0.218	0.105	0.128	"	0.353	0.346	0.007	61
NN 4	0.248	0.232	0.105	0.124	"	0.353	0.356	0.003	58

Summary of Results.

Ca Expressed as mg. per 100 c.c.

Normal Human.		Normal Dog.		Human Rickets.		Dog Tetany.	
Serum Ca.	Per Cent. Diffusible Ca, %.	Serum Ca.	Per Cent. Diffusible Ca, %.	Serum Ca.	Per Cent. Diffusible Ca, %.	Serum Ca.	Per Cent. Diffusible Ca, %.
10.5	65	10.9	68	9.0	68	6.3	62
11.1	67	11.1	69	7.6	63	6.1	63
10.5	67	10.7	60			6.7	66
10.3	68	10.6	61				
11.0	72	10.2	69				
10.4	70						

These observations indicate that there is no change in the proportion of diffusible serum calcium in human rickets, or in experimental dog tetany.

138 (1720)

The relation of acid base equilibrium in the body to excretion of phosphorus and calcium.

By **T. F. ZUCKER.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*]

The relation of the acid base equilibrium in the animal body to calcium and phosphorus metabolism has been studied by a number of workers.[1] The significance of variation in the acid base equilibrium is undoubted, but considerable confusion is encountered, when an attempt is made to correlate the available results. A good many of the experiments have been done on small animals (rabbits), so that very large doses of acid, for instance, had to be given to obtain effects marked enough to study analytically. Repeated doses of 75 c.c., .9 per cent. HCl given to the rabbits of 1.5 kg. is very considerable and in the experiments of Fitz, Alsberg and Henderson usually finally resulted in death of the animal. Under such conditions, secondary results due to the large amount of acid must have complicated the picture.

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[1] Gerhard and Schlesinger, *Arch. exp. Path. u. Pharm.*, 1899, xlii, 83.
Fitz, Alsberg and Henderson, *Am. Jour. Phys.*, 1907, xviii, 113.
Schabod, *Arch. f. Kinderh.*, 1909, lii, 47; 1910, liii, 381; 1911, liv, 83.
Graustrӧm, *Ztschr. f. physiol. Chemie*, 1908, lviii, 195.
Goto, *Jour. Biol. Chem.*, 1918, xxxvi, 355.
Givens, *Jour. Biol. Chem.*, 1917, xxxi, 421.
Ruedel, *Arch. f. exp. Path. u. Pharm.*, 1894, xxxiii, 79.
Dubois and Stolte, *Jahrb. Kinderh.*, 1913, xxxiii, 21.
Nelson and Williams, *Jour. Biol. Chem.*, 1917, xxviii, 231.

This paper is an attempt to formulate the relation of acid base equilibrium to the urinary and fecal Ca and P excretion in normal adult man on a uniform mixed diet in which the greater portion of P and Ca are given in an easily available form. Three experiments were done on the effect of administration of HCl and $NaHCO_3$, the one recorded here showing the effect of base and acid successively on the same diet. Previous to the days recorded in the tables, the subject had been on the diet for four days. The assumption is made that he was in calcium and phosphorus equilibrium, which is verified by the totals shown in the second table. The experiment was divided into three periods: normal, basic and acid. The P_H of the urine in the normal period was 5.7 to 5.9 and the effect of administration of base and acid is seen clearly. The acidity (titrated according to Folin) runs parallel to this. The ammonia nitrogen shows clearly the well known effect of acid and base administration. The urinary P and Ca are progressively diminished by the $NaHCO_3$ and progressively increased by the acid.

Now on turning to the second table, which gives figures for urine and feces for periods constituting the last three days of the periods as given in the first table, we find that the total Ca and total P excreted in each period is constant. It is hardly to be expected that either loss or storage should occur under these conditions, sufficient to be discernible in a three day period. A dose of acid comparable to 75 c.c. of .9 per cent. HCl for a rabbit would undoubtedly produce a negative Ca balance. It may be noted that the CO_2 combining power of the blood during the entire experiment remained within the range of normal. In the alkaline period it rose to 77.7 and in the acid period went down to 69. Normal was 74.

The very close agreement in total Ca and P between periods in this experiment is accidental, the other experiments showing a somewhat greater variation. It is seen that in the second period, while the total P and Ca is the same as in period I, its distribution between urine and feces is changed. In the normal period, 57 per cent. of P and 28.7 per cent. of Ca are found in the urine, while in the alkaline period, these figures are 44 per cent. and 22 per cent. respectively. The acid period shows a reversal of this change, more Ca and P appearing in the urine.

Considering the fact that as high as 70 per cent. of the ash of feces is made up of Ca and P it seems quite plausible that an equilibrium condition should exist between the calcium phosphates in the intestine and the Ca and P of the blood and tissues which is influenced by the relative amounts of acid and base being metabolized. That Ca and P, with regard to absorption of reexcretion in the intestine, play a role entirely different from the other mineral elements is apparent from analyses of intestinal contents at various stages of digestion and the feces.[2]

Fitz, Alsberg and Henderson assumed that the increased phosphate in the urine after acid administration came from the tissues. This undoubtedly is true with extreme acidosis, but the data here recorded seem to show that urinary Ca and P can be increased at the expense of the fecal Ca and P, without loss to the body, if the acidosis is mild. A moderate dose of $NaHCO_3$ may produce the reverse effect, without storage of Ca or P, in the body.

Besides the general biochemical significance which these data have, their application to nutritional studies and such problems as rickets should not be overlooked. The Ca and P excreted in the feces may be considered not available to the tissues and since the relative amount of acid and base in the diet influences the loss of Ca and P in the feces, the same level of intake may have a different nutritional significance, depending on the acid base equilibrium. The latter may be influenced not only by preponderance of acid or base forming elements in the food, but also by metabolic acidosis or alkalosis or possibly even by the intestinal bacterial flora.

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[2] For data see Schmidt and Strassburger, "Die Fäces des Menschen," or Schreuer in Oppenheimer's Handbuch, Band III, 2.

Further experiments are in progress to determine the limits of acid and base administration, in which the above observations hold, and to extend the study to various levels of Ca and P intake.

TABLE I.

Period.	Date Nov.	Vol. c.c.	D.	P_h.	NH_3 gm.	Acidity c.c. N/10	P gm.	Ca gm.
I. Normal........	14	960	1.020	5.8	1.42	726	2.06	.394
	15	1620	1.021	5.9	.91	748	1.66	.376
	16	1320	1.023	5.7	1.20	815	1.99	.455
	17	1225	1.025	5.8	1.20	760	1.71	.446
II. 15 gm. $NaHCO_3$.	18	1950	1.019	6.5	.52	400	1.79	.389
	19	1850	1.021	7.2	.28	120	1.32	.307
	20	1700	1.022	7.3	.21	160	1.41	.297
III. 300 c.c. N/10HCl	21	1480	1.024	5.7	.61	508	1.68	.385
	22	1075	1.027	5.3	1.09	800	1.84	.467
	23	1250	1.023	4.9	1.44	850	1.86	.435
	24	1320	1.022	4.9	1.65	787	1.90	.512

TABLE II.

3 Last Days of Period.	P in Urine.	P in Feces.	Total P.	Per Cent. in Urine.	Ca in Urine.	Ca in Feces.	Total Ca.	Per Cent. in Urine.
I. Normal..........	5.36	3.99	9.35	57	1.27	3.17	4.44	28.7
II. 15 gm. $NaHCO_3$...	4.52	4.72	9.24	44	.99	3.42	4.41	22.2
III. 300 c.c. N/10 HCl..	5.60	3.53	9.13	61	1.41	2.94	4.35	32.4

139 (1721)

Studies on experimental rickets, IV. Cod liver oil as contrasted with butter fat in the protection against the effects of insufficient calcium in the diet.

By E. V. McCOLLUM, NINA SIMMONDS, P. G. SHIPLEY
and E. A. PARK.

[*From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, and from the Department of Pediatrics, Johns Hopkins University, Baltimore.*]

In our experimental work we have made observations which demonstrate in a striking way the differences in the effectiveness of cod liver oil as contrasted with butter fat in influencing the rate and extent of growth, and their effects on the histological structure of the bones. This is well illustrated by the results of restricting young rats to the following diet:

Wheat	30.0
Maize	19.5
Rice (polished)	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak	10.0
$NaCl$	1.0
$NaHCO_3$	1.5
Cod liver oil	3.0

This diet induces fairly good growth and fertility during at least 8 to 10 months, notwithstanding its deficiency in calcium. The calcium content of this food mixture is 0.059 gms. per 100. The optimum calcium content of this diet is reached when about 1.5 per cent. of calcium carbonate is added. The phosphorus content of this mixture is 0.3546 gms. per 100, and is not far from the optimum content. This food mixture is slightly alkaline owing to its content of sodium bicarbonate.

This food mixture with butter fat to the extent of 3, 10, or 20 per cent. of the food mixture fails to induce an appreciable amount of growth. With 20 per cent. of butter fat the animals gain but a few grams in weight, look very inferior, become short and stocky, and are rough coated. They usually die within a few months.

This diet when 10 per cent. of casein is added, but without the addition of either butter fat or cod liver oil, causes pathological changes characteristic of rickets. With small amounts of cod liver oil (3 per cent.) no rachitic changes are seen in the bones. Even 20 per cent. of butter fat fails to effectively direct the growth processes in the bones toward the normal condition.

This food mixture containing 3 per cent. of butter fat and 1.5 per cent. of calcium carbonate is a highly satisfactory diet for the promotion of growth to the full adult size, the maintenance of high fertility throughout the breeding period in the females and the successful nutrition of the young by the mothers. Even a fifth generation in a family confined to this diet showed no signs of physical deterioration. Without the calcium addition, but with 3 per cent. of butter fat, very little growth can take place.

The striking differences between the nutritive properties of cod liver oil as contrasted with butter fat we have up to the present time been able to demonstrate in so satisfactory a manner only with diets in which the phosphorus content is approximately the optimum, and with the calcium content distinctly below the optimum. The results of feeding these two fats with a diet similar to that described but in which the phosphorus content is low and the calcium varies from low to high, we shall discuss in a forthcoming paper.

140 (1722)

Studies on experimental rickets, V. The production of rickets by means of a diet faulty in only two respects.

By **P. G. SHIPLEY, E. A. PARK, E. V. McCOLLUM** and **NINA SIMMONDS.**

[*From the Department of Pediatrics, and from the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.*]

The following diet, when fed to the young rat, in a comparatively short time (three to five weeks) produces rickets. The diet is composed of:

Rolled oats	40.0
Gelatin	10.0
Wheat glutin	7.0
Sodium chloride	1.0
Potassium chloride	1.0
Calcium carbonate	2.0
Dextrin	39.0

This diet is extremely poor in fat-soluble A, the anti-xerophthalmic substance. Young rats develop xerophthalmia when placed upon it in from four to five weeks. Its proteins are of good quality and are supplied in abundance (21 per cent.). The phosphorus content is relatively low (0.209 gms. per 100 gms. of the food mixture). The calcium content is approximately the optimum.

The gross evidences of rickets in the rats eating the faulty ration were briefly these: deformities of the thorax consisting in flattening or hollowing along the line of costochondral junctions; pigeon breast; enlargement and distortion of the costochondral junctions; fractures of the ribs; enlargements of the ends of all the long bones; diminished resistance of the bone to cutting; great diminution in the tensile strength; and finally, the presence of a zone of a white or pale yellow color, between the cartilage and the shaft, visible to the naked eye, the rachitic metaphysis.

The microscopic evidences of rickets in the skeleton of the rats receiving the diet were convincing. There was increased thickness and great irregularity of the proliferative cartilage which extended in irregular prolongations toward the shaft, complete absence of calcium deposition in the cartilage or great defects in calcification, the presence of an intermediate zone between cartilage and shaft, the zone already alluded to as the rachitic metaphysis, composed of cartilage in all stages in the process of metaplasia into osteoid, osteoid trabeculæ, blood vessels accompanied by marrow elements, irregular deposits of calcified intercellular substance encased in osteoid and, finally, by broad osteoid investments of the trabeculæ of the shaft. The condition in the bone produced by the faulty diet in question may be said to have been at all points identical with that seen in advanced rachitis of human beings.

When now butter fat was added to the faulty diet just discussed in the proportion of 0.5 per cent. of the total ration, the occurrence of the xerophthalmia was somewhat postponed and the life of the animal lengthened. The pathological condition of the skeleton, however, remained essentially unmodified. If anything, it was intensified, probably as the result of the slight stimulation given by the butter fat to the growth of the bone.

When butter fat was added in the proportion of 10 per cent. of the total ration, the occurrence of the ophthalmia was effectually prevented, life was prolonged for a number of months and the pathological condition in the skeleton was somewhat modified but still remained rickets (observations on four rats). In one animal, light but uniform calcification in the zone of cartilage nearest the shaft was present. In all four rats the metaphysis was exceedingly deep and composed in greater part of interlacing osteoid trabeculæ separated by blood vessels, the cartilage exceedingly irregular, the trabeculæ of the shaft very numerous and surrounded by osteoid. Even the addition to the faulty ration of a large quantity of butter fat did not, therefore, prevent the development of the rickets.

When, on the other hand, cod liver oil was added to the diet in the proportion of 2 per cent. of the total ration, no pathological condition of any sort developed in the skeleton. The addition of cod liver oil to the faulty diet, therefore, completely prevented the development in the skeleton, not only of changes of a rachitic nature but of all changes of a pathological nature.

When the faulty diet was still further modified by the subtraction of the added calcium carbonate, so that calcium was deficient as well as the phosphorus and the organic factor, a pathological condition developed in the skeleton which, however, was not rickets.

When the calcium carbonate of the faulty diet was replaced by 2 per cent. $CaHPO_4$, so that the diet was deficient in the organic factor alone, the pathological condition which developed in the skeleton was not rickets but osteoporosis.

Conclusions: 1. When the content of the diet in the anti-rachitic organic factor or factors is sufficiently low, the protein of the diet and the quantities of Mg, Na, K, Cl, Fe and I being optimal or nearly optimal, a disproportion in the quantities of calcium and phosphorus present of such nature that the calcium is in optimal or above the optimal quantity but the phosphorus low, gives rise to rickets.

2. When the level of the phosphorus in the faulty diet is raised, so that both calcium and phosphorus are present in presumably adequate amounts, and the fault in the diet, therefore, becomes limited to a deficiency in the organic factor, a rachitic condition does not develop. A deficiency in the organic factor alone does not produce rickets, as our experiments have attested with other diets.

3. When no calcium carbonate is added to the diet, so that the quantities of both calcium and phosphorus are low, rachitis does not develop.

4. Butter fat is feeble in its anti-rachitic properties. This fact, repeatedly confirmed in our experiments, is in our opinion of

great practical importance in so far as the explanation of the occurrence of rickets in the human being is concerned.

5. Cod liver oil, on the contrary, as our experiments have repeatedly shown, is powerful in its anti-rachitic properties.

141 (1723)

Subcutaneous tubes for chemotactic studies and leucocyte collection.

By **FREDERICK L. GATES.**

[*From the Rockefeller Institute for Medical Research, New York City.*]

For the study of the chemotactic influences of various substances, including bacteria and their products, and for the collection of phagocytes and other wandering cells, small glass test tubes of about 5 c.c. capacity have been placed in the loose subcutaneous tissues of the rabbit. The procedure is as follows.

The mouths of test tubes, 1.2 × 5 cm., are covered with drum heads of fine bolting silk, tied on with thread, which permit free interchange of liquids with the subcutaneous tissues and the migration of cells and bacteria. The chemotactic material or the organism to be studied, either in agar (1 c.c.) overlaid with Ringer's solution, or in fluid form is then added to the sterilized tubes through a hollow needle. A tube may be placed in each flank through a single dorsal incision, after blunt dissection of the loose subcutaneous tissue. Surgical anesthesia and asepsis are required for the operation. Removal of the tube contents is accomplished by hypodermic puncture through the cloth heads of the tubes, after careful cleansing of the skin with iodine and alcohol. The needles of two 20 c.c. syringes are plunged into the tube. One syringe is already filled with Ringer's solution or other liquidto replace the tube contents as it is drawn into the second syringe. Thus the tube is washed out and left full of fresh material. The tubes themselves are practically non-irritating and their contents may be recovered daily, or at longer intervals, for weeks or months.

From such tubes, containing a small amount of aleuronat in agar and filled up with Ringer's solution, leucocytes for phagocytic experiments may be collected daily over a long period. During the first day few leucocytes enter the tubes, but after 48 hours, and subsequently, a 15–20 c.c. specimen of ground glass opacity may be recovered from each tube. Practically all of these cells are polymorphonuclear leucocytes and about 80 per cent. of them are viable, as observed in a hanging drop in which the dead cells are stained with aqueous carmine or trypan blue. Of the living cells, 15 per cent. or less will die during the 24 hours after removal, indicating that 65–75 per cent. of the cells are in good condition, an observation which is confirmed by their phagocytic activity.

The introduction of bacteria into the tubes furnishes an opportunity for the observation of certain phases of the struggle between invader and host. In general, the method seems applicable to a variety of studies in chemotaxis and resistance to invasion.

142 (1724)

Experiences with thyroidectomy and ligation of the thyroid arterie in depancreatized dogs.

By G. A. FRIEDMAN and J. GOTTESMAN.

[*From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University, New York.*]

The absence of glycosuria and the high sugar tolerance in myxoedematous individuals led us to undertake the experiments cited in this brief report.

Dog No. 100, male, weight 14 kilo., partial pancreatectomy performed January 12, 1921. Urine positive for sugar the following day. It persisted in amounts of from 2 to 3 per cent. until January 19, when both inferior thyroid arteries were ligated. Glycosuria continued until January 26, 1921, when complete thyroidectomy was done, sparing the parathyroids. Urine became negative for sugar on the following day and continued so. On February 5, 1921, with blood sugar 95 mgms. an additional 3.4

gms. of pancreatic tissue was removed with no change in the urinary findings. On February 9, blood sugar was 86 mgms. On this day 150 gms. glucose were introduced by the stomach tube. Tests for urinary sugar remained negative. On May 9, rest of the pancreas, amounting to 10 gms. was removed. Urine continued negative for sugar until May 14, when intestines prolapsed through wound, followed by death next day. Autopsy revealed complete absence of pancreatic tissue. His blood sugar was 66 mgms. before the last pancreatectomy and his weight 15.9 kilo.

Dog No. 106, male, weight 7.52 kilo., complete pancreatectomy on March 2, 1921; weight of removed pancreas being 18.2 gms. Urine positive for sugar, beginning day following operation in amounts varying between 5 and 6 per cent. On March 5, both superior and inferior thyroid arteries were ligated. He continued to have sugar in decreasing amounts until March 8, when the urine became negative for sugar. Urine continued to be negative for sugar until March 19, when distemper developed followed by death the next day.

Blood sugar findings were as follows:

March 2, before operation	76	mgms.	
" 4, after pancreatectomy	250	"	
" 5, after ligation	192	"	
" 6	90	"	
" 7	50	"	unreadable.

Autopsy revealed lobular pneumonia involving all the lobes. Complete absence of pancreatic tissue.

Dog No. 111, male, weight 6.66 kilo., complete pancreatectomy done on April 4, 1921. Weight of removed specimen 17 grams. Blood sugar prior to operation 78 mgms. Urine positive for sugar on day following the operation. Blood sugar on April 10, 200 mgms. On this day the right superior and inferior thyroid arteries were ligated. Urine continued positive for sugar until April 17, when the thyroid arteries on the left side were ligated. Blood sugar on this day was 244 mgms. No change in the urinary sugar until April 19, on which day death occurred from tetany. Weight before death 5.66 kilos.

Diet of all the animals was liberal consisting of meat, bread and water. All wounds healed per primam. Further experiments along these lines are in progress.

143 (1725)

Experimental studies of the pharmacology of quinidin.

By **ALFRED E. COHN** and **ROBERT L. LEVY.**

[*From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.*]

It has recently been shown that in certain patients suffering from fibrillation of the auricles, the normal cardiac mechanism may be restored by the oral administration of quinidin sulphate. Knowledge concerning the pharmacological action of this drug is desirable for the clinic and is important insofar as it may furnish information concerning the nature of the fibrillatory process.

Experiments were done on 12 dogs. Anesthesia was accomplished by intratracheal etherization. The thorax was opened in the median line. A myocardiograph of the Roy and Adami type was sewed into the tip and base of the ventricles and connected with a system of Marey tambours. Changes in the degree of cardiac contraction obtained by this method may be taken to represent alterations in volume output. The blood pressure was recorded from the right femoral artery; injections of the drug were made into the left femoral vein. In eight experiments the threshold for the production of transient auricular fibrillation was determined by faradization of the right auricle. Electrocardiograms were made at frequent intervals.

The results may be summarized as follows:

1. *Rate.*—No constant effect was noted. There was acceleration 4 times, slowing 4 times and fluctuation in the remainder of the animals.

2. *P-R* (*conduction*) *Time.*—The changes were slight and relatively insignificant. There was prolongation four times and shortening once.

3. *T-wave.*—A change in this portion of the electrocardiogram occurred eight times. This consisted either of reversal in the direction of the deflection, or of increase in the voltage of the original wave.

4. *Blood Pressure.*—In all of the animals a striking fall in blood pressure was observed, the extent of the fall depending in a measure on the amount of the drug injected. There was partial, but never complete return to the former pressure level.

5. *Degree of Contraction.*—There was always an increase in the height of the stroke recorded by the lever, the increase ranging from 16 to 162 per cent. A point was always reached after which further introduction of the drug caused diminution in volume output. The increase in cardiac contraction occurred simultaneously with the fall in blood pressure, but persisted and often continued after partial restoration of the blood pressure level. Unlike the augmentation accompanying the fall in pressure induced by histamine or hemorrhage, the increase produced by quinidin is usually due to greater muscle shortening, not to diastolic relaxation. The persistence of effect likewise indicates a direct stimulating action on the heart muscle. The relation of the fall of pressure to the height of contraction is, however, not finally determined by these experiments.

6. *Threshold for the Production of Auricular Fibrillation by Faradization.*—In four experiments this was slightly raised; in four others, there was no demonstrable change.

7. *Effect on Premature Contractions.*—In one animal frequent spontaneous ventricular premature contractions were noted. These disappeared after the injection of quinidin.

8. *Mode of Death.*—Only one animal died with ventricular fibrillation. In the others, there was progressive slowing of the heart, sometimes with the occurrence of sino-auricular block. The auricles, as a rule, ceased before the ventricles. In the final curves were seen isolated, orderly ventricular beats.

9. *Lethal Dose.*—The lethal dose (mg. per kg.) was extremely variable. In general, the greater the fractionation of dosage, the greater was the amount of drug necessary to cause death.

Experiments dealing with the effect of quinidin on the rate of conduction of the excitation wave through heart muscle are in progress.

144 (1726)

Hydrogen ions, titration and the buffer index of bacteriological media.

By J. HOWARD BROWN.

[*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.*]

The titration of bacteriological media should not be regarded as a crude method of determining the reaction of media, but a process which reveals facts not disclosed by a simple hydrogen ion determination. For many puposes a knowledge of the buffer content of media is quite as important as the hydrogen ion concentration. The importance of the buffer content of media has been indicated by Kligler,[1] Bermann and Rettger,[2] Bronfenbrenner and Schlesinger,[3] H. Jones,[4] L. F. Foster[5] and C. G. L. Wolf.[6]

The buffer content of media between stated limits of hydrogen on concentration is easily determined by titration against a standard acid or alkali solution. The amount of alkali required to reduce the hydrogen ion concentration of a medium from its initial reaction to a stated lower hydrogen ion concentration, say P_H 8.0, may be called the "reserve acidity"[7] of the medium indicated by the symbols $R_H(P_Hn - 8)$ in which n = the initial P_H. The amount of acid required to raise the hydrogen ion concentration from P_Hn to, say, P_H 5.0 may be called the "reserve alkalinity"[7] indicated by the symbols $R_{OH}(P_Hn - 5)$. The "buffer index" indicated by the symbols $BI(P_H8 - 5)$ is the sum of the reserve acidity plus the reserve alkalinity. Each of these values is to be expressed in terms of per cent. normal acid or alkali, *i.e.*, the number of cubic centimeters of $N/1$ acid or alkali required to change the hydrogen ion concentration of 100 c.c. of medium from one stated hydrogen ion concentration to the other. While for most purposes of interest to the sanitary or medical bacteriologist the range of hydrogen ion concentration between the limits of P_H 8.0 and P_H 5.0 is sufficient, the buffer index between other limits may be determined for special purposes.

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[1] I. J. Kligler, *J. Bact.*, 1916, i, 663.

[2] N. Bermann and L. F. Rettger, *J. Bact.*, 1918, iii, 389.

[3] J. Bronfenbrenner and M. J. Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1918, xvi, 44.

[4] H. M. Jones, *J. Inf. Dis.*, 1920, xxvii, 169.

[5] L. F. Foster, *J. Bact.*, 1921, vi, 161.

[6] C. G. L. Wolf, *Brit. J. Exp. Path.*, 1920, i, 288.

[7] E. W. Washburn, *Proc. 2nd Meeting Ill. Water Supply Assn.*, 1910, p. 93.

The titration of a large number of samples of bouillon of supposedly the same composition showed wide variation in their buffer indices. If one is working with an easily cultivated organism such as *Bacterium coli* and wishes to determine its limiting hydrogen ion concentration in a few hours, a medium of low buffer index should be selected. If on the other hand a large amount of growth or the fermentation of a large amount of sugar is desired, a medium of high buffer index should be used. Less fermentable sugar is required in a poorly buffered medium than in a medium of high buffer content. In a bouillon of low buffer index a small amount of dextrose may be sufficient to produce a high terminal acidity whereas the same organism may ferment a much larger amount of dextrose in a bouillon of high reserve alkalinity and high buffer index and yet produce a terminal alkalinity.

The author has devised a very simple method of titrating the reserve acidity, reserve alkalinity, and buffer index of media, a method requiring only a few cubic centimeters of medium and easily carried out by a laboratory technician in a few minutes. A description of this method is now in press.

145 (1727)

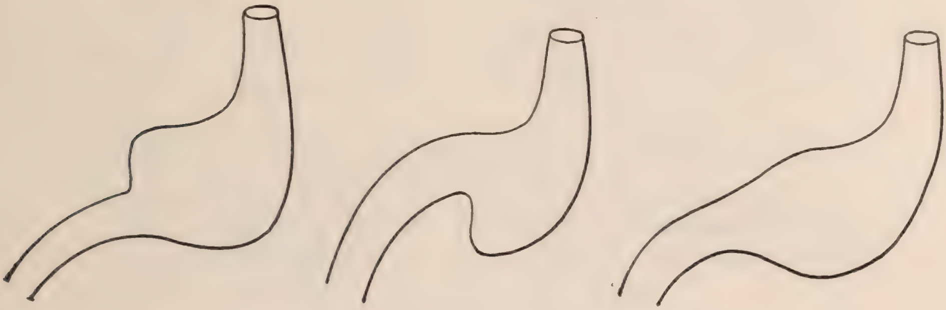
I. Gastric resection: experimental data on the duodenal loop.

By **W. HOWARD BARBER** and **LOUIS C. LANGE.**

[From the Department of Experimental Surgery, New York University and Bellevue Hospital Medical College.]

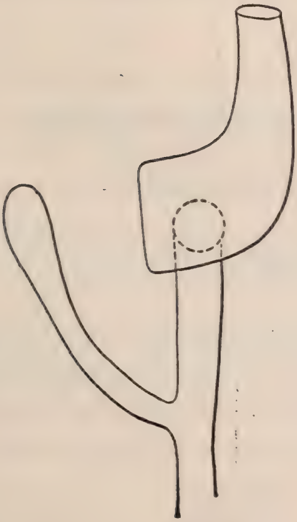
Operable new growths and malignant ulcers require in selected cases resection of the pyloric end of the stomach. After resection, the surgeon is forced to meet the problem of gastroenterostomy.

Taking for granted the removal of the greater portion of the pyloric end of the stomach, continuity of the gastroenteric canal may be re-ëstablished by any one of the following methods as suggested in the following diagrams:



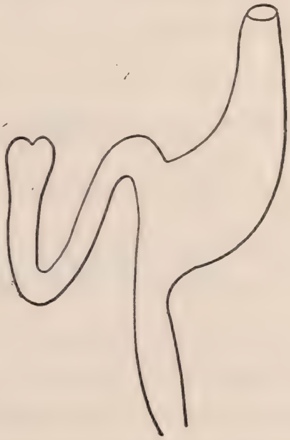
A *B* *C*

Terminal Gastroduodenal Anastomosis.



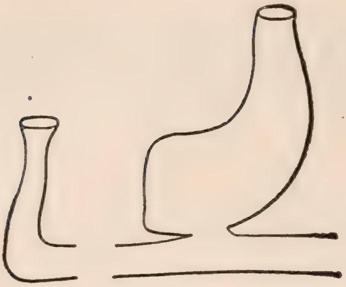
D

Lateroterminal Gastrojejunal Anastomosis.

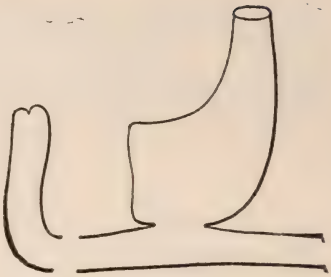


E

Terminolateral Gastrojejunal Anastomosis.



F



G

Lateral Gastrojejunal Anastomosis.

Compatible with life.	*Incompatible with life.*
Terminal methods, *A*, *B*, *C*.	Lateroterminal, *D*.
Lateral, *F*.	Terminolateral, *E*.
	Lateral, *G*.

Lethal Factor-Pancreatic Injury.

Types of gastroenteric anastomosis following partial gastrectomy.

These experiments have been performed upon dogs without mortality in the method of terminal gastroduodenostomy and with high mortality in those methods involving duodenal occlusion or duodenostomy.

146 (1728)

II. Gastric resection: notes on the surgical pathology.

By **W. HOWARD BARBER** and **LUIGI CELANO.**

[*From the Department of Experimental Surgery, New York University and Bellevue Hospital Medical College.*]

Terminal anastomosis between the divided ends of the stomach and jejunum, as represented in Part I, Fig. D, combined with duodenal occlusion and implantation of the duodenal loop into the jejunum caudad of the stomach gives the following results:

Exp. No.	Days of Life.	Pathology.
69	4	Peritonitis.
77	2	Pancreatitis.
82	3	Pancreatitis.
83	2	Peritonitis.
84	3	Pancreatitis.
85	2	Pancreatitis.
91	1	Pancreatitis.

Lateral anastomosis *without resection* but with occlusions of the pyloric end of the stomach and duodenum Fig. G show the following results:

Exp. No.	Days of Life.	Pathology.
201	2	Pancreatitis, superficial necrosis and erosions in stomach and intestine about stoma.
207	3	Necrosis similar to but earlier than in 201.
219	1	Pancreatitis.

Lateral anastomosis combined with simple ligation in first portion of duodenum:

Exps. 86 and 87 after 6 and 2 days, respectively, pancreatitis.

Lateral anastomosis without resection but with pyloric occlusion and duodenostomy, Fig. F:

Exp. No.	Days of Life.	Pathology.
204	5	Pancreatitis.
205	4	Pancreatitis.
209	2	Pancreatitis; hydropneumothorax.
210	3	Pancreatitis.
211	14	Malnutrition.
213	14	Ulcer, gastrojejunal.

In the above experiments, mobilization of the extreme oral end of the duodenum in the dog sufficient for closure and inversion of that end is invariably followed by fatality of evident pancreatic origin. Of six duodenostomies, four give evidence of pancreatitis and two (211, 213) show extreme emaciation on fourteenth days.

Note: In the above tables, the term pancreatitis has been used to designate the complex found in the clinical cases: fat necrosis, free hæmolyzed blood in the peritoneal cavity and often in the intestinal loops, and evidences of pancreatic injury from congestion and cloudy swelling to suppuration.

147 (1729)

Studies in the physiology of vitamins. II, Does vitamin-B stimulate glands in a manner similar to the alkaloid pilocarpine?

By GEORGE R. COWGILL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

The hypothesis[1] that vitamin-B functions to stimulate glands in a manner similar to the alkaloid pilocarpine has been investigated experimentally. Extracts of rice polishings, wheat embryo, navy bean and yeast, and neutralized tomato juice, all of which were demonstrated to contain vitamin-B by tests on polyneuritic animals (pigeons and dogs), were examined for their action on the secretory function of the salivary glands. The effect of intravenous injection of these products on the flow of saliva was noted in anesthetized dogs in which the ducts of the submaxillary and sublingual glands were cannulized. In order to ascertain whether any slight temporary flow of saliva that might follow the injection was due to a vaso-dilator effect of the injected product on the sympathetic nervous system, blood pressure was determined by means of a manometer connected with the femoral artery. Normal dogs and polyneuritic dogs were used.

All of these products gave negative results. Stimulation of the chorda tympani nerve or injection of pilocarpine, however, always produced a characteristic flow.

148 (1730)

A contribution to the study of the relationship between vitamin-B and the food intake in the dog.

By GEORGE R. COWGILL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

Karr[2] showed that in the dog some relationship exists between the desire to partake of food and the amount of vitamin-B which is ingested. Yeast and tomato juice were used as sources of vitamin-B in his experiments. The increase of appetite which followed the administration of these products was believed to be due to the vitamin-B contained therein since yeast appeared to be less potent in this respect when autoclaved. This conclusion is supported by the results obtained in our experiments in which extracts of rice polishings, wheat embryo and navy bean were tested for the property of promoting appetite in dogs which had been fed on a diet lacking this dietary essential. The administration of any one of these preparations to such a dog was followed by a recovery of appetite which lasted for varying periods. All of these products were demonstrated to contain vitamin-B by tests on polyneuritic animals (pigeons and dogs). The potency of these products in promoting appetite seemed to parallel their potency in relieving symptoms in polyneuritic animals and this parallelism suggests that vitamin-B is the appetite-promoting factor in the preparations used.

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[1] Uhlmann, *Zeitsch. f. Biol.*, 1918, lxviii, 3.

[2] Karr, *Jour. Biol. Chem.*, 1920, xlviv, 255.

149 (1731)

Further studies on the affinity of sheep-corpuscles for anti-sheep hemolysin.

By **R. L. KAHN** and **D. S. LYON.**

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

In previous studies[1] on the rate of absorption of anti-sheep hemolysin by sheep corpuscles, it was shown that 0.05 c.c. of packed sheep-cells added to a saline solution containing 400 units of hemolysin, will absorb as many as 390 units after 10 minutes extraction at room temperature. The hemolysin was obtained by immunizing rabbits with sheep-cells in the usual manner and a unit was taken to be the smallest quantity which hemolyzed 0.1 c.c. of a 5 per cent. suspension of packed sheep-cells in the presence of 0.1 c.c. (2 units) of pooled guinea-pig complement after 15 minutes incubation in the water-bath. It was observed also that extraction periods of 5, 10, 15 and 20 minutes did not show any marked differences in the number of hemolysin units extracted by the corpuscles. A study of the effect of temperature on the rapidity of hemolysin extraction, indicated but a small increase in the number of units extracted at water-bath as compared with ice-box temperature.

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[1] Kahn, R. L., and Lyon, D. S., *Proceed. Soc. of Amer. Bacter.*, Lansing Branch, 1920; *Abst. of Bacter.*, 1921, v, 23.

With this data on hand, a quantitative study was undertaken of the hemolysin absorption capacity of 0.05 c.c. of packed sheep-cells when employing different concentrations of hemolysin. Accordingly 0.05 c.c. quantities of centrifugalized cells were added to a series of tubes each containing different quantities of hemolysin in 1 c.c. quantities of saline. The extractions were carried out for 10 minutes at room-temperature, after which period the cells were thrown down by rapid centrifugation and the number of unabsorbed hemolysin units remaining in the supernatant fluid, determined. The following table gives the results obtained in one of a series of such experiments.

TABLE SHOWING THE EFFECT OF THE CONCENTRATION OF HEMOLYSIN ON THE ABSORPTION CAPACITY OF 0.05 C.C. OF PACKED SHEEP-CELLS.

Tube No.	No. of Units Per c.c.		No. of Units in Supernatant fluid.	No. of Units Extracted.
1	14,300	0.05 c.c. packed sheep-cells added to each tube and extraction permitted for 10 minutes at room-temperature, followed by rapid centrifugation.	5,550	8,750
2	10,725		2,500	8,225
3	7,150		1,250	5,900
4	3,575		199	3,376
5	2,860		150	2,710
6	2,145		67	2,078
7	1,430		33	1,397
8	715		10	705
9	357		1	356
10	178		0	178

The marked affinity of sheep-cells for anti-sheep hemolysin is well illustrated in this table. In similar studies by Morgenroth and Arrhenius,[2] an hour extraction period was employed, while the quantity of sheep-cells used for extraction is not stated.

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[2] Arrhenius, S., "Immunochemistry," 1907, p. 150.

The next step undertaken was to determine the stability of the sheep-cell-hemolysin union. This is of practical importance inasmuch as many workers "sensitize" sheep-cells by placing a mixture of cells and hemolysin for 1 hour in the water-bath, while our studies suggested the possibility of dissociation of cells and hemolysin during this period.

Our findings indicate that there is little difference between the quantity of hemolysin absorbed after 10 minutes extraction at room-temperature as compared with 1 hour extraction in the water-bath. In a few cases, less extraction was observed at the latter time and temperature compared with the short extraction period at room temperature. There are two probable explanations for this finding; first, the dissociation of cells and hemolysin after prolonged exposure at warm temperature; second, the hemolysis of a small number of corpuscles due to agitation, causing some absorbed hemolysin to get back into solution.

150 (1732)

The effect of heat on specific complement fixing antibodies.

By R. L. KAHN and S. R. JOHNSON.

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

The comparative thermostability of specific complement fixing antibodies resulting from protein immunization, and thermolability of so called complement fixing antibodies present in syphilitic sera, was recently reported by one of us[1] in these PROCEEDINGS. The former antibodies were found to withstand a temperature of 70 degrees C., while the latter were destroyed between 62 and 65 degrees C. In view of the fact that more and more interest is being developed in complement fixation in connection with bacterial diseases, these studies were extended to complement fixing antibodies resulting from bacterial immunization.

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[1] Kahn, R. L., PROCEED. SOC. EXP. BIOL. AND MED., 1921, xviii, 171.

Three antisera were employed: (1) Antityphoid (rabbit) serum, (2) antiabortion (ox) serum and (3) antimallei (rabbit) serum. The antigens were bacillary suspensions of the specific organisms titrated in accordance with standard technique. The complement fixation tests were carried out in each case with unheated serum and the same immune serum subjected to different temperatures up to 85 degrees C., for varying intervals.

It was found after subjecting these sera to a water-bath temperature of 65 degrees C. (the thermal destructive temperature of syphilitic sera) for 1 hour, that the antibodies remained in tact. Higher temperatures showed varying degrees of antibody destruction depending particularly on the time of exposure. One hour heating at 70 degrees C. caused between 20 and 60 per cent. of antibody destruction. Thirty minutes exposure at 75 degrees C. caused between 80 to 90 per cent. of destruction. Fifteen minutes at 80 degrees C. destroyed the antibodies completely.

151 (1733)

On the persistence of complement fixing antibodies in the serum of rabbits immunized with purified proteins.

By **R. L. KAHN.**

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

This report is based on two experiments: Rabbit A was immunized intravenously with edestin and Rabbit B, intraperitoneally with phaseolin. Rabbit A received the first injection of protein November 15, 1920, and the last, eight days later. The quantities injected were 50, 75, 100, 125 and 150 mgm.—a total of 0.5 gm. Rabbit B received its injections between December 28, 1920, and January 5, 1921; the quantities were 100, 150, 200, 250 and 300 mgm. of protein—a total of 1 gm. The sera of these rabbits were examined from time to time for the presence of specific complement fixing antibodies, the last examination having been made on May 5, 1921. The results showed a gradual decrease in the number of antibodies. A sufficient number of these bodies, however, were present during the May determination to merit the designation of 3 plus (+++) with the usual complement fixation technique.

The case of Rabbit A, immunized with 0.5 gm. of protein showing in its blood the presence of complement fixing antibodies five months after immunization, is of significance inasmuch as it revives the old disputed question as to the nature of the complement fixing antibody. The widely accepted view that this antibody is an indication of the presence of an active antigenic manifestation in the body as differentiated from the agglutinin, for example, which is a true antibody, is brought to question ; since one would have to assume that some of this small quantity of protein is present in the animal in some form, after 5 months—a quite unlikely condition.

152 (1734)

Influence of radium and x-rays on the frog's leucocytes.

By **M. M. STURGES** and **ISAAC LEVIN.**

[*From the Department of Cancer Research, Montefiore Hospital, New York.*]

The white blood corpuscles are the most sensitive cells to the action of radium and x-rays. The senior writer has indicated in his previous publications that this action differs specifically for the various types of the white blood cells. This "selective" biological action of the rays goes even beyond the apparent structural differences of the cells. The rays for instance destroy rapidly the lymphocytes of lymphatic leukemia, while they have a comparatively slight effect on the lymphocytes in conditions of inflammatory leucocytosis. As a general rule the result of the action of radium and x-rays on the normal blood consists in the diminution of the number of lymphocytes and a relative increase in the number of the polymorphonuclear leucocytes. The other types of leucocytes usually remain unaffected.

The real significance of this phenomenon will remain obscured until a clearer insight is gained into the derivation and the comparative functional significance of the two types of white blood cells.

The numerical proportion of the lymphocytes and the polymorphonuclear leucocytes differs in the various animal species and it is therefore of great importance for the ultimate elucidation of the whole problem to test the action of the rays on different species of animals.

The present investigation consisted in subjecting to the action of radium and x-rays normal frogs and also frogs in whom a change in the white blood cells was induced by a preliminary injection of yeast.

X-raying of Normal Frogs.—The method consisted in taking a total and differential blood count of the animal before the raying. The whole animal was then x-rayed (45 minutes, Coolidge tube, 7 ma. 9-inch spark gap, 5-inch focal distance) and blood counts taken at various intervals for four days. The results obtained were as follows. The total leucocyte count showed practically no difference from the normal count before radiation, and the same holds true for the other series of experiments. The differential count showed a marked change in the numerical relationship between the polymorphonuclears and lymphocytes, while the number of the eosinophiles and transitionals remained practically stationary. To cite an instance,—2 eosin., 14 poly., 84 lympho. changed into 2 eosin., 70 poly., 28 lympho.

This change was most marked 24 hours after radiation, and the blood usually became normal after about four days.

Radiumization of Normal Frogs.—The method consisted in the introduction into dorsal lymphsac of a frog of a minute capillary glass tube about 4 mm. long containing from 1.0 to 0.6 millicuries of radium emanation. This method produces a slow and continuous action of the rays of radium on the organization of the animal. The results obtained on the blood were quite analogous to those produced by the x-rays. The important difference, however, consisted in the fact that the numerical difference between the lymphocytes was most pronounced only about three days after the insertion of the radium emanation capillary. For instance, the normal blood showed 1 eosin., 1 poly., 98 lympho.: 24 hours after radiumization, 2 eosin., 34 poly., 64 lympho., and 72 hours after radiumization 1 eosin., 85 poly., 13 lympho.

Radiumization of Yeasted Frogs.—The experiments consisted in the injection of an emulsion of yeast into a normal frog, and this was followed 24 hours later by x-raying the animal or an insertion of a radium emanation capillary. The injection of yeast is followed by a change in the blood of a frog similar to the one induced by the x-rays or radium, and the change is most marked 24 hours after the injection and continues for a few days. For instance, the normal blood showed 0 eosin., 18 poly., 82 lympho. 23 hours after yeasting, 1 eosin., 73 poly., 26 lympho. Now the remarkable phenomenon observed in this series consisted in the fact that neither the x-rays nor the radium produced any further noticeable change in the numerical relationship between the lymphocytes and the polymorphonuclear leucocytes, or at the most a very slight additional decrease of lymphocytes. To cite an instance: the normal blood showed 2 eosin., 22 poly., 76 lympho.: 17 hours after yeasting, 0 eosin., 60 poly., 49 lympho. The animal was then x-rayed and immediately after showed 3 eosin., 68 poly., 29 lympho.: 24 hours later, 1 eosin., 80 poly., 19 lympho.

The analysis of the experiments shows that in the frog, as in the human, the action of the rays consists mainly in the diminution of the relative number of lymphocytes. However in the normal frog it does not seem to be accompanied by a noticeable change in the total blood count. The radiations seem to produce a different effect on lymphocytes of a normal frog from the one produced on the lymphocytes of a yeasted frog. This phenomenon is also analogous to effects which are related in the beginning of the paper as occurring in the human.

153 (1735)

The cure of infantile rickets by artificial light and by sunlight.

By ALFRED F. HESS and LESTER J. UNGER.

[*From the Home for Hebrew Infants, New York.*]

Some years ago we reported an attempt to cure rickets in infants by means of the ultra-violet rays. The results were not conclusive. Since this time Huldschensky has reported cures by this method. During the past winter this therapeutic measure was again employed and its effect followed by means of frequent radiographs of the epiphyses of the long bones. The mercury-vapor lamp was used and exposures of the entire body were made every few days from three to twenty minutes at a distance of 120 to 75 cm. The effect in all cases was curative, as demonstrated both by clinical examination and by the appearance of calcification at the ends of the bones.

Following this success with artificial rays the effect of sunlight on infantile rickets was investigated. To this end infants were exposed, under careful supervision, to the sun's rays in increasing degree. After a period of three to four weeks a similar calcification of the epiphyses was noted, as well as general improvement. This beneficial effect of the sun's rays as well as of the artificial rays was achieved although the diet was in no way altered; some of the babies were receiving, both preceding and during the treatment, dry milk of the same lot.

These results lead to the conclusion that the remarkable seasonal incidence of rickets is due to the seasonal variation of sunlight; that many cases of rickets are due to defective hygiene rather than to dietary errors (although diet is also an etiologic factor in this disorder); that sunlight should be used to prevent and to cure infantile rickets; and that in metabolism studies both on animals and on man, the influence of sunlight must be noted and taken into account.

154 (1736)

Cholesterol and cholesterol esters in blood showing a positive Wassermann reaction.[1]

By **ARTHUR KNUDSON, THOMAS ORDWAY** and **HAZEL FERGUSON.**

[*From the Departments of Medicine and Biological Chemistry, Albany Medical College and Albany Hospital.*]

Blood was taken from a series of patients having various forms of syphilis and showing a strongly positive Wassermann reaction. Total cholesterol and cholesterol esters were determined on the whole blood by the method of Bloor,[2] and Bloor and Knudson,[3] respectively. The syphilitic cases having a positive reaction were grouped into syphilis of the heart and blood vessels, syphilis of the central nervous system, syphilis with skin manifestations. Several cases with positive Wassermann reaction but showing no physical signs and control cases with negative Wassermann reaction were also studied. The results of these determinations are given in Table I. They indicate that the total cholesterol content is not effected but the amount of cholesterol as esters is considerably decreased in the various syphilitic conditions. The percentage of cholesterol ester in the normal patients with negative Wassermann reaction averages 34.4 per cent. which agrees very closely with the results reported by Bloor and Knudson.[4] The per cent. of cholesterol esters in the cases with syphilis showing positive Wassermann reaction and distinct physical signs averages about 21–22 per cent. It is of interest to note that the three cases with a positive Wassermann reaction but no physical signs of syphilis do not show a very marked reduction of the cholesterol esters.

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[1] The researches reported in this investigation were supported by a grant from the United States Interdepartmental Social Hygiene Board.

[2] Bloor, W. R., *Jour. Biol. Chem.*, 1916, xxiv, 227; *ibid.*, 1917, xxix, 437.

[3] Bloor, W. R., and Knudson, A., *Jour. Biol. Chem.*, 1916, xxvii, 107.

[4] Bloor, W. R., and Knudson, A., *Jour. Biol. Chem.*, 1917, xxix, 7.

TABLE I.

CHOLESTEROL AND CHOLESTEROL ESTERS IN HUMAN BLOOD.
Mg. per 100 c.c.

Case No.	Wassermann Reaction.	Total.	As Esters.		Diagnosis.
		mg.	mg.	Per Cent.	
2	negative	190	70	36.8	Normal.
8	negative	223	76	34.0	"
20	negative	166	58	34.9	"
139	negative	183	69	37.7	"
138	negative	196	62	31.0	"
Average.				34.8	
213	++++	215	47	21.8	Syphilis of heart and blood vessels.
102	++++	143	25	17.4	" " " " " "
119	++++	178	46	25.8	" " " " " "
103	++++	143	29	20.2	" " " " " "
104	++++	174	36	20.6	" " " " " "
118	++++	223	46	20.6	" " " " " "
116	++	192	59	30.6	" " " " " "
207	++++	190	47	24.7	" " " " " "
Average.				22.7	
203	++++	175	46	26.2	Syphilis of central nervous system.
166	++++	208	44	21.1	" " " " "
106	+++	217	55	24.2	" " " " "
137	++++	196	35	17.8	" " " " "
141	++++	192	22	11.4	" " " " "
Average.				20.1	
208	++++	208	44	21.2	Syphilis with skin manifestations.
38	++++	234	60	25.6	" " " "
179	+++	185	31	16.7	" " " "
Average.				21.2	
202	++++	230	82	35.6	No physical signs of syphilis.
204	++++	139	36	28.5	" " " " "
212	++++	190	58	30.5	" " " " "
Average.				31.5	

The cholesterol and cholesterol ester content has also been studied in a series of rabbits' blood before and after they have developed a four positive Wassermann reaction as a result of experimental inoculation with spirochete pallida. The results of these experiments are given in Table II. Of the seven rabbits studied all but one show a marked reduction in the amount of cholesterol esters. The average percentage of cholesterol combined as esters in the rabbits before inoculation is 34.3 per cent. and after developing a positive Wassermann reaction it has dropped to an average of 22.9 per cent. These results are in close agreement with the determination on the human cases.

The balance between cholesterol and its esters is very significant in both of these experiments. In no other disease so far reported in the literature has such an altered relation between bound and free cholesterol been observed. No conclusion as to the significance of these results can be drawn as yet but it may be possible that they are bound up in the question as to the nature of the Wassermann reaction.

TABLE II.

CHOLESTEROL AND CHOLESTEROL ESTERS IN RABBIT BLOOD.
Mg. per 100 c.c.

Rabbit Number.	Wassermann Reaction Negative.			After Developing four Positive Wassermann Reaction.		
	Total.	As Esters.		Total.	As Esters.	
	mg.	mg.	Per cent.	mg.	mg.	Per cent.
470	102	36	35.2	99	24	22.2
471	111	39	35.1	117	15	12.8
472	93	33	35.4	117	19	16.1
473	96	25	26.0	98	27	27.5
474	92	30	32.6	93	26	27.9
475	114	45	39.7	90	21	23.2
476	85	33	36.0	87	27	31.0
Average.			34.3			22.9

155 (1737)

On the elimination of the x-chromosome from the egg of drosophila melanogaster by x-rays.

By **JAMES W. MAVOR.**

[*From the Department of Biology, Union College, Schenectady, N. Y.*]

The experiments to be described were performed with a view to determining if x-rays by affecting the x-chromosome could disturb the inheritance of a sex-linked character. Wild type (red-eyed) females of *Drosophila melanogaster*, homozygous for red-eye were x-rayed soon after emerging from the pupa with a dose just under the sterilization dose and mated to white-eyed males. The white-eyed character being recessive to the red-eyed the normal result of crossing a homezygous red-eyed female with a white-eyed male is for the offspring in the first generation to be all red-eyed if there is no non-disjunction.

In all, four experiments have been completed to date. In three of these thirty-five virgin females, homozygous for the red-eyed character, were mated with white-eyed males. Nineteen of these were used as controls and sixteen were x-rayed soon after emerging from the pupa and immediately before mating. The rayed females were the sisters of the controls. *None of the nineteen control pairs produced white-eyed males.* One of the rayed females was sterile. *Of the fifteen fertile, rayed females, twelve produced one or more white-eyed males.*

In two of the four experiments the white-eyed males which were crossed with the rayed females were also homozygous for dumpy, a second chromosome character. All of the six exceptional males produced had normal wings with the exception of one which died before its wings expanded. It therefore seems probable that only the x-chromosome was affected.

Further experiments using multiple sex-linked stock, eosin miniature and scute-echinus-cut-vermilion-garnet-forked, have shown that probably the whole x-chromosome is affected.

Since the eggs which produced the exceptional males were laid during the first six days after raying it seems reasonable to believe that they were acted upon while in or preparing for one of the maturation divisions. Further, since the x-chromosome behaves differently from the other chromosomes during these divisions the production of the exceptional males would be accounted for if it is assumed that the x-rays were applied to the egg cells from which they came at a time when the x-chromosome in them was in a condition particularly susceptible to the dose of x-rays given.

156 (1738)

The survival value of conjugation in the life history of Spathidium spathula.

By **LORANDE LOSS WOODRUFF** and **HOPE SPENCER.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

In a former communication it has been shown that in our pedigree cultures of *Spathidium spathula*, exconjugant lines, in the great majority of cases, exhibit a higher rate of division during the first fifteen days than the parent lines.[1] The purpose of the present paper is to summarize briefly the results to date with respect to the effects of conjugation as exhibited later in the life history of the pedigree lines.

1. Conjugation in the majority of cases increases the length of life of the exconjugant line, so that it lives after the death of the parent so-called "non-conjugant" line. This is shown by the fact that of forty-seven exconjugant lines, thirty lines lived longer; four lines lived to essentially the same date; and thirteen lines died before their respective parent lines.

2. The total number of generations attained by the exconjugant exceeds those attained by the parent from the date when the exconjugant arose to the death of the parent in about eighty per cent. of the lines. Of fifty-two comparable lines, forty-one lines exceeded their respective parent lines in number of fissions; two practically equalled; and nine lines did not attain so many generations. The fact is evident that conjugation increases the number of fissions to a total which could not otherwise have been reached.

3. If the period be considered during which both lines were alive, the results are even more conclusive. Forty-four lines attained more generations than the parent; four equalled the parent; and four completed a smaller number of fissions. Thus over eighty per cent. of the exconjugant lines attained more generations than their respective parent lines.

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[1] L. L. Woodruff and Hope Spencer, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 240–241.

4. By analysis of the data from an entirely different angle the same general conclusion is apparent. The total number of generations attained before the F_1 generation appeared, plus those from the F_1 to the origin of the F_2, and so on to the F_5 generation, amounts to date to from 450 to 550 generations, according to the series followed. The first line reached 234 generations without conjugation, therefore by conjugation it thus far has been possible more than to double the number of fissions obtainable without conjugation.

In brief, all the data thus far secured indicate that in this pedigree culture, under the conditions of the experiment, the survival value of conjugation in the majority of cases is marked—the exconjugant lines exhibiting a higher division rate and outliving the non-conjugant lines.

The complete paper will appear in the Journal of Experimental Zoology.

157 (1739)

The precipitation of botulinus toxin with alcohol.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From The Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

While attempting to purify botulinus toxin by precipitation with ethyl alcohol, we found that the alcohol causes destruction of this toxin. Even a weak solution (20–30 per cent.) of ethyl alcohol is capable of quickly destroying many thousand lethal doses of botulinus toxin in vitro. This destruction takes only five to ten minutes if the mixture is kept at 37° C. On the other hand the toxins of tetanus and diphtheria are much more resistant to the destructive effects of alcohol.

It had been observed in several outbreaks of botulism that those who had partaken freely of alcoholic beverages while eating the incriminated food were not severely affected or remained entirely well, whereas others eating as much or even less of the same food were severely and sometimes fatally poisoned.

We assumed that this protection from botulinus poisoning when alcoholic beverages are partaken might be due to the direct destructive action of the ethyl alcohol on the toxin in the stomach. The problem was approached experimentally. Two series of three guinea pigs each of equal weight received each *per os* enough botulinus toxin to kill them within 24 to 48 hours. Each guinea pig of one of the series received 6 c.c. of 30 per cent. alcohol *per os* immediately following the toxin. The guinea pigs of the first series died within the usual time, whereas the animals which received the alcohol survived and are apparently normal two weeks after the experiment.

The effect of alcohol must be ascribed solely to its direct destructive action upon botulinus toxin. This action is quite unlike etherization[1] which delays the rate of absorption of botulinus toxin. That alcohol destroys botulinus toxin only by direct action is shown by the following experiments: Guinea pigs receiving botulinus toxin by the mouth die within the usual incubation period observed in normal controls despite the administration of large amounts of alcohol subcutaneously. The same fatal results are seen when the toxin is given intraperitoneally and the alcohol administered by the mouth.

We are at present studying the question of the effect of stomach contents as well as quantitative and time relation necessary to assure the destructive action of alcohol upon toxin in the stomach. We are also studying the question of the mechanism of this destructive action of alcohol with the view of finding satisfactory substitutes for it.

(This work is a part of the investigation of food poisoning, conducted under the Direction of Dr. M. J. Rosenau, professor of preventive medicine and hygiene, Harvard Medical School. The investigations were made under the auspices of the Advisory Committee of the National Research Council on the Toxicity of Preserved Foods, and under a grant to Harvard University from the National Canners Association.)

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[1] J. Bronfenbrenner and H. Weiss, PROC. SOC. EXP. BIOL. AND MED., 1921, xxiii, 253.

158 (1740)

The lactic acid in the blood of dogs in exercise.

By **A. BAIRD HASTINGS.**

[*From the Department of Physiology, Columbia University, New York.*]

In the course of our study of fatigue, it became desirable to make determinations of the concentration of lactic acid in the blood. Ryffel's method was used with the modification that the formaldehyde standard was tested against a known lactic acid solution in each experiment. The blood was obtained from the jugular vein of dogs which were exercised on a motor-driven treadmill. The results of typical experiments are given in Tables I and II.

It will be seen from Table I that severe exercise of short duration brought about a marked increase in the concentration of the lactic acid in the blood. The results given in Table II, however, show that when the exercise was of moderate intensity and long continued, the concentration of lactic acid in the blood was decreased. We believe that the increase of the lactic acid in the blood in the initial stages of severe exercise may be associated with the hyperpnea occurring at that time, and that its subsequent decrease, due perhaps to an increased efficiency of the oxidative processes, may be related to "second wind." In view of the fact that no increase has been found in the lactic acid in exercise however long continued, its significance as a primary factor in physiological fatigue, not carried to exhaustion, would seem open to question.

TABLE I.

THE EFFECT OF SEVERE EXERCISE OF SHORT DURATION ON THE CONCENTRATION OF LACTIC ACID IN THE BLOOD OF DOGS.

Dog.	Date.	Distance in Miles.	Rate in Miles/hr.	Lactic Acid in Mgm./100 c.c.	
				Before Exercise.	After Exercise.
A	March 10, 1921	0.28	9.3	5.3	17.3
B	February 9, 1921	0.24	8.0	12.4	19.9
C	March 16, 1921	0.23	7.7	11.7	55.3

Table II.

The Effect of Moderate Exercise, Long Continued, on the Concentration of Lactic Acid in the Blood of Dogs.

Dog.	Date.	Distance in Miles.	Rate in Miles/hr.	Lactic Acid in Mgm./100 c.c.	
				Before Exercise.	After Exercise.
A......	February 7, 1921.....	11.0	4.9	4.9	2.9
	February 15, 1921....	27.0	4.5	7.8	6.2
B......	February 9, 1921.....	10.8	5.0	12.4	6.3
	May 3, 1921.........	5.2	5.2	24.2	16.7
C......	January 30, 1921.....	13.7	5.5	13.4	9.6
	February 24, 1921....	22.8	4.6	13.2	7.8

159 (1741)

A case of metastatic calcification associated with chronic nephritis and hyperplasia of the parathyroids.

By **ROGER S. HUBBARD** and **JOHN A. WENTWORTH.**

[*From the Clifton Springs Sanitarium, N. Y.*]

The case was one of severe chronic nephritis in a man of twenty years. About the larger joints of the upper extremities and about the feet were many fluctuant tumor masses of various size, and distributed along the course of many of the peripheral arteries were innumerable bead-like nodules which could be felt, while x-ray plates showed extensive calcification of the peripheral arteries and large irregular deposits about the joints.

The aspirated contents of one of the tumors was found to be a thick, creamy fluid simulating pus in the gross, containing amorphous material without cells and a suspension of calcium phosphate of 30 per cent. Otherwise the analysis of the fluid resembled normal synovia.

A peripheral artery bearing the nodules was excised from the leg and was found to be stiff, very tortuous, and to have numerous small yellow and white deposits of calcium in the wall. Microscopically the media was largely replaced by calcium salts, the intima was much thickened, and in places there was little lumen.

Only limited metabolic studies were possible because of the patient's serious condition. Urinary and fecal calcium determinations were done but are of no value as enemata were necessary for constipation. On an ordinary low protein diet, with little milk, the blood calcium was high, being 13.4 mgs. per 100 c.c. of blood, fell to 11.9 mgs. after three days of a low calcium diet, and rose to 12.7 mgs. on a high calcium diet for three days.

The other blood analyses showed the usual findings of a severe progressive, chronic nephritis with a marked lowering of the alkaline reserve.

The patient died 11 months after the first tumor masses appeared, and autopsy revealed a severe interstitial nephritis with a right hydronephrosis, calcium deposits in the wall of the left auricle, extensive and marked calcification of the smaller peripheral arteries, the arteries of the gastro-epiploic omentum, and the jejunum, but sparing the liver, spleen, lungs, kidneys and stomach, an osteitis fibrosa particularly marked in the skull, ribs and vertebræ, and two large parathyroid bodies, 2 c.m. in diameter, with some hyperplasia microscopically and a small adenoma in one.

Hyperplasia of the parathyroids has been described in a few cases of chronic nephritis but there appears to be no description of such extensive calcium changes as this case presents, except in metastatic calcification, and this man did not show the deposits in the lungs, kidneys and stomach, usually found in the latter condition.

160 (1742)

Studies on atmospheric requirements of bacteria.
I. Water vapor tension.

By NICHOLAS KOPELOFF and STERNE MORSE.

[*From the Research Laboratories of the Psychiatric Institute, of The New York State Hospitals, Ward's Island, N. Y.*]

Quantitative experiments on the relation between the growth of bacteria and the amount of water present have been limited to studies on the effect of water content of media, and are surprisingly meager in the amount and character of the work done. Such investigations are limited to those of Wolf[1] and Weigert,[2] who working with different concentrations of media found definite limits of growth for various organisms. No investigation as to the effect of water vapor tension on surface colonies on a solid medium has apparently ever been made.

The method used for obtaining preliminary data as to the influence of atmospheric moisture on surface growth consisted in using either two tops or two bottoms of ordinary petri dishes which are placed edge to edge and held together with adhesive tape around the entire circumference giving practically an air-tight capsule. In the upper half, media was poured to a depth of 2 mm.; in the lower half, solutions of various dehydrating agents were placed to a depth of 5 mm.; about 2 cm., as a rule separating the two surfaces. 1/10 c.c. of a dilution of broth culture of the organism investigated, was spread on the surface of the poured medium and evenly distributed with a bent rod over the entire surface.

The organisms investigated were *B. coli*, *B. subtilis*, *Staphylococcus aureus*, and *Streptococcus hemolyticus*. The medium used was glucose infusion agar, P_H 7.0, and cultures were usually incubated 18 hours at 37.5° C. The dehydrating solutions used were glycerin, 50 per cent. glycerin, 50 per cent. calcium chloride (saturated) and 25 per cent. calcium chloride, giving initial relative humidities of 0, 25, 35, and 75 per cent. respectively at the start of the experiment. The rate of change of the water vapor tension with addition of water, of the glycerin solutions is, however, so much larger than that of the calcium chloride solutions, that with the conditions as above stated the drying action of the calcium chloride is usually somewhat more energetic than that of the glycerin.

Marked inhibition of growth of all organisms investigated was shown to take place under these conditions, the action being greater on the more delicate organisms and roughly proportional to the extent of the dehydration which occurs. This phenomenon is obvious in colonies as soon as they can be distinguished by the microscope, *i.e.*, before extensive drying of the medium has occurred. The characters and appearance of all colonies investigated were profoundly modified by a change in this variable. Marked variation from controls over water occurs with the solutions used having a water vapor tension of 75 to 85 per cent. relative humidity, thus showing that a relatively slight departure from saturated conditions is of considerable importance in bacterial surface cultures.

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[1] Wolf, L., *Archiv. f. Hyg.*, 1896, xxxiv, 200–209.

[2] Weigert, R., *Centbl. f. Bakt.*, I, Orig., 1904, xxxvi, 112–121.

It might be added that the simple device above described serves excellently for anaërobic plate cultures, with alkaline pyrogallate solution in place of the dehydrating solutions, dextrose-methylene blue media being easily decolorized and remaining so.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

Twenty-ninth meeting.

Berkeley, California, May 4, 1921.

161 (1743)

Councilmania lafleuri, a new amœba of the human intestine.

By **CHARLES ATWOOD KOFOID** and **OLIVE SWEZY.**

[*From the Zoölogical Laboratory, University of California, and the Bureau of Communicable Diseases, California State Board of Health.*]

There exists some confusion and uncertainty among investigators of human intestinal amœbæ as to certain features of structure and activities of the non-pathogenic *Endamœba coli* (Loesch) Schaudinn. The points in debate are its habit of ingesting red blood corpuscles, its mobility in the free state, the existence of more than one type of cyst, the structure and location of the karyosome, the presence of cysts with an irregular number of nuclei (other than 2, 4, 8, 16), and the escape of amœbulæ from the cysts in fæces.

We believe these questions have arisen from the confusion hitherto by investigators, of two species, *Endamœba coli* and a new one, generically distinct, which we designate as *Councilmania* *lafleuri* in honor of the two investigators who first clearly demonstrated the pathogenicity of the amœba which they called *Amœba dysenteriæ* and which Schaudinn, in ignorance of their work, later named *Entamœba histolytica.*

This new amœba was found by us in stools wholly free from *Endamœba dysenteriæ,* especially in blood and mucus strands, during sixteen weeks almost continuous daily examinations. It was present in large numbers on every day but three, and free stages were obtained in liquid stools, especially in mucus, and strands of cellular tissue therein. Continued intestinal disturbance accompanied this case of infection which occurred in a returned soldier from overseas who had been four months in the hospital in France with dysentery and had had two treatments here for *Endamœba dysenteriæ,* with emetin bismuth iodide, the last with salvarsan treatment also. After the second treatment this amœba entirely disappeared. Five other cases of infection have been observed by us. Amœbæ which are probably *Councilmania* appear in the figures of Casagrandi and Barbagallo (1897), Prowazek (1911), Werner (in Prowazek's "Handbuch") (1911), Walker and Sellards (1913), Mathis and Mercier (1917), Clauri (1917), and possibly elsewhere. It is probably widely distributed as a human parasite.

In the free stage it has survived for five hours in the mobile condition in the thermos bottle. It is extraordinarily mobile, throws out perfectly hyaline, broadly rounded, single pseudopodia with expulsive suddenness and travels rapidly through obstacles. Its cytoplasm is gorged with food vacuoles including bacteria, and cysts of *Chilomastix.* In the mucus strands it was frequently filled with red blood corpuscles.

In the encysted stage it runs through 1-, 2-, 4-, and 8-cell stages. We have seen one 12-cell cyst. Most of the cysts, except in liquid stools, are in the 8-cell stage when discharged in the fæces.

The cysts are exceptionally thick-walled, are double-contoured, tend to be ellipsoidal or spheroidal rather than spherical, and range from 11 to 34 μ, generally 16–20 μ, in longest diameter. Some of the cysts are found to have a chromophile protoplasmic ridge which eventually pierces the cyst wall and forms the avenue of escape of the small amœbulæ. This may occur in the fæces at the time of discharge or even later.

The structure of the nucleus differs from that in *E. coli.* The cysts are more difficult to stain. In the cysts the nuclear membrane is lightly encrusted with granular chromatin, the central karyosome is large, often asymmetrical or reniform, and in the prophase is broken up into more or less distinct granules. It is less often excentric than in *E. coli.* The interzonal area between the karysome and nuclear wall stains lightly, if at all, in iron hæmatoxylin. There are eight chromosomes at the metaphase, while *E. coli* has six. An intradesmose forms within the nucleus on the nuclear membrane at mitosis between the daughter centrosomes which form deeply stained massive polar caps.

The chromatoidal bodies are often massed in the early stages, are splinter-like or thread-like, and pointed, and are rarely found after the four-cell stage, except as rounding-up bodies. They show a greater tendency to mass than in *E. coli.*

The "glycogen" vacuole is present in the one to four-cell stages. It is spheroidal, central, and lobed as it disappears. It does not stain brown in iodine as in *E. coli,* though the cyst as a whole stains yellow. It does not give the typical glycogen reaction in Best's carmine. Otherwise in occurrence and behavior it resembles the glycogen body of *E. coli.*

As a result of bud formation and the discharge of amœbulæ one finds cysts with varying numbers of nuclei. We have seen from three to twelve. The latter number appears to result from nuclear multiplication during the period of escape of the amœbulæ. We have never seen this phenomenon of the ridge-like bud and the successive discharge of amœbulæ in *E. coli.* This budding of amœbulæ and the structure of the nucleus in the cysts distinguish *Councilmania* from *Endamœba* and *Endolimax.* *Councilmania* appears to be pathogenic, but more evidence is needed on this point. It occurs also in carriers.

162 (1744)

Botulism; a study of the action of the toxin of B. botulinus upon the living tissues.

By **ERNEST C. DICKSON** and **RICHARD SHEVKY.**

[From Stanford University Medical School, San Francisco.]

In a study of the action of the toxin of *B. botulinus* upon the living body it was found that the upper and lower neurones of the skeletal motor nerve supply are unimpaired and that there is no disturbance in the spinal reflex arcs of the extremities. There is, however, definite evidence of a blocking of the nerve impulses in the nerves of the non-sympathetic portion of the involuntary nervous system, *e.g.*, the vagus nerve, the chorda tympani and the nervi erigentes. There is indication that this blocking is of a temporary and relatively unstable nature, and that it is not due to organic destruction of the nerve elements.

Cats, dogs and rabbits were used in the experiments.

163 (1745)

The agglutinating action of salvarsan in vitro and in vivo.

By **JEAN OLIVER** and **SOSABRO YAMADA.**

[From the Department of Pathology, Stanford University Medical School, San Francisco.]

A detailed study of the mechanism of the agglutinating action of salvarsan on red blood cells shows the following points.

In vitro salvarsan has a fairly constant titre of agglutination. There is a progressive drop in this titre as the solution is oxidized on standing. Although the red cells have the power to bind salvarsan in isotonic sugar solutions, no agglutination takes place unless a certain amount of salt is added. Serum, as well as other hydrophilic colloids, under proper conditions may prevent the occurrence of agglutination. It was shown that this inhibition was due to a prevention of the union of salvarsan with the cells.

Salvarsan injected intravenously into rabbits in large doses causes intravascular agglutination which may be observed in the drawn blood. The lungs of such animals are filled with petechial hemorrhages resulting from emboli of the agglutinated red cells, and the animals die acutely.

A certain proportion of animals injected at three-day intervals with sub-lethal doses die during the third or fourth injection in a manner resembling the death of animals injected with large doses. In these animals also the anatomical evidences of intravascular agglutination and pulmonary embolism are constantly present.

The total sum of these repeated doses barely reaches the concentration found necessary for immediate intravascular agglutination, and as this total sum must be considerably reduced by the excretion of salvarsan during the six or nine days intervening, some additional factor must be responsible for the sudden occurrence of intravascular agglutination in these animals.

Examination of their blood shows no increase in the agglutinability of the red cells during the course of the injections, but there is a distinct drop, in some cases to one-sixteenth of the original value, in the power of the serum to inhibit, in vitro, the agglutination of red cells by salvarsan.

164 (1746)

Impure and misnamed stock cultures of obligate anaërobes.

By IVAN C. HALL.

[*From the Department of Bacteriology and Experimental Pathology, University of California.*]

During the past few years the writer has engaged in building up a collection of cultures of obligate anaërobes whose purity and true identity should be, if possible, beyond question. In addition to numerous cultures isolated from original sources a number have been received from other laboratories. Most of these were already labelled as to species and form the subject of this report.

While a few cultures were received with aërobic contamination these are not included here since aërobic contaminations are readily recognized and eliminated by the bacteriostatic action of selective dyes[1] or by selective heating according to whether the contaminants form spores or not.

No assumptions as to purity or correct identity were made; after ascertaining the absence of aërobes, every culture was first carefully examined for the salient properties of the indicated species in order to determine, (1), its presence or absence, and (2), the possible presence of contaminating organisms. Irrespective of the findings in the preliminary tests, every culture was regarded as possibly impure and was therefore purified and repurified from three to six times by either the deep colony, or the surface colony method or both.[2] The pure culture was then identified.

Following are the results briefly tabulated.

More than one species of anaërobe (6 cultures)	
Containing the designated species	5 cultures
Not containing the designated species	1 "
No evidence of anaërobic impurity (40 cultures)	
Renamed in accord with recently accepted nomenclature	7 cultures
Incorrectly labelled	13 "
Correctly labelled	20 "
Total	46 cultures

This experience indicates that the majority of anaërobic cultures received from other laboratories are pure but that a surprisingly large number contain or consist of resistant species not indicated by their labels. *B. sporogenes* is the commonest organism found in stock cultures of anaërobes.

The writer believes that many of the cultures were pure and properly identified in the beginning of their history which in some cases goes back many years and involves transfers between several laboratories. The most probable source of anaërobic contamination is imperfectly sterilized culture medium. Once contaminated a culture may readily lose the possibly less resistant species indicated by its label while the contaminant may persist indefinitely without detection by any but one skilled in anaërobic bacteriology.

A few instances have been observed in our own laboratory in which pure cultures of anaërobes became contaminated and the original species completely supplanted by another. Cultures of anaërobes require exceptional care to avoid contamination and subsequent loss of identity; no culture can be accepted upon its face value.

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[1] Hall, "Selective elimination of hay bacillus from cultures of obligative anaërobes," *Jour. A. M. A.*, 1919, lxxii, 274.

[2] Hall, "Practical methods in the purification of obligate anaërobes," *Jour. Infec. Dis.*, 1920, xxvii, 576.

165 (1747)

The variation in the size of trypanosoma brucei according to the host.

By **T. D. BECKWITH** and **W. W. REICH.**

[*From the Department of Bacteriology and Experimental Pathology, University of California.*]

It has been stated by observers that a certain species of trypanosome may show differences in size according to the host infected. An examination of the literature however reveals much divergence of opinion concerning this matter. In addition such statements as appear are very fragmentary. The technique upon which some of them are based moreover leaves much to be desired.

Plimmer and Bradford[1] (1898) quoted by Castellani and Chalmers remark that the length of *Trypanosoma brucei* is constant for a given animal but varies in different hosts, being between 26 and 27 micra in rats, mice, guinea pigs, rabbits and dogs. Kanthack, Durham and Blandford[2] state that the Nagana parasites vary considerably both in size and in form. Bruce, Hamerton and Bateman[3] come to the conclusion that *Trypanosoma brucei* varies from 10 to 16 micra in length in the rat with an average of 13.0 while in guinea pigs the limits are 8 to 16 micra with the average 12.5. Laveran and Mesnil[4] claim that their own work which included parasites from a large range of mammals shows no manifest variation in size of the organism.

A culture of *Trypanosoma brucei* was obtained from Dr. F. G. Novy at the University of Michigan. Immediately upon receipt, it was suspended in sterile physiological saline and injected subcutaneously into a white rat. In order that some carefully collected data might be acquired concerning possible variations in the size of the micro-organism in the rat and in the guinea pig, blood from this animal very shortly after death was injected into a guinea pig. Heart blood from this guinea pig at the time of its autopsy in turn was injected into another guinea pig and also into a white rat. By this method the host altered from time to time. The series of animals included four rats and four guinea pigs, all of which showed no parasites before the time of use. Numerous slide preparations were made from heart blood as soon after death as possible, within an hour or two at most. These were stained with Wright's stain made up according to the usual formula but diluted with one additional equal part of methyl alcohol. Measurements were then made by means of a properly calibrated series of lenses and with a filar micrometer. Slides made from each animal were included. By this means were examined 100 individuals from the guinea pigs and 90 from the rats. All trypanosomes measured were in the fully developed stage with typical morphology.

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[1] Plimmer and Bradford, quoted from Castellani and Chalmers, "Manual of Tropical Medicine," 3d edition, 1920.

[2] Kanthack, Durham and Blandford, *Proc. Roy. Soc. London*, 1908, lxiv, 100.

[3] Bruce, Hamerton and Bateman, *Proc. Roy. Soc. London*, 1909, lxxxi, 14.

[4] Laveran and Mesnil, "Trypanosomes et Trypanosomiases," 1912.

As a result it is found that the average size of *Trypanosoma brucei* in guinea pigs is 18.46 × 3.16 micra while in white rats it is 13.25 × 2.23 micra. The application of statistical methods shows that these two series are comparable since deviations for guinea pig trypanosomes are—length 13.68 and width 4.27 while for white rats they are—length 14.27 and width 3.59. Coefficients of dispersion are: guinea pigs—lengths 0.13 and breadth 0.23; for white rats they are determined to be—length 0.18 and breadth 0.27. This variation in the two hosts was apparent throughout the period of active infection.

We find therefore that *Trypanosoma brucei* does vary in size according to the host infected and that it is markedly larger in the guinea pig than in the white rat. These findings are comparable to those of Hegner[5] with *Trypanosoma diemyctyli* in salamanders.

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[5] Hegner, *Jour. Parasit.*, 1921, vii, 105.

RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

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Gates, Frederick L.

1623. Preparation of collodion sacs for use in bacteriology.

1624. [with **Peter K. Olitsky.**] Factors influencing anaerobiosis with special reference to the use of fresh tissue.

1723. Subcutaneous tubes for chemotactic studies and leucocyte collection.

Gay, F. P.

1603. [with **Bernice Rhodes.**] Experimental bronchopneumonia and empyema in the rabbit.

Geiling, E. M. K.

1677. [with **H. H. Green.**] Studies in the regeneration of blood.

Gibbons, Marion.

1635. See **Little, C. C.**

Gie s,M. H.

1648. [with **H. B. McClugage** and **E. G. VanHorne.**] The antiscorbutic property of raw, dried and cooked apples and bananas.

1659. [with **Harry B. McClugage.**] The effect of heat and age upon the antiscorbutic vitamine in tomatoes.

Gortner, R. A.

1632. See **Harris, J. A.**

Gottesman, J.

1724. See **Friedman, G. A.**

Gratia, Andre.

1678. Studies on the lytic agent of Bordet and Ciuca.

1691. Preliminary report on a staphylococcus bacteriophage.

Green, H. H.

1677. See **Geiling, E. M. K.**

Greenwald, Isidor.

1696. On the supposed relation between alkalosis and tetany and similar conditions.

Hadjopoulos, L. G.

1681. See **Kahn, Max.**

Hall, Ivan.

1746. Impure and misnamed stock cultures of obligate anaerobes.

Harris, J. Arthur.

1584. Formulæ for the determination of the correlations of size and growth of increments in the developing organism.

1626. [with **A. T. Valentine.**] The specific electrical conductivity of the tissue fluids of desert Loranthaceæ.

1632. [with **R. A. Gortner, W. F. Hofman** and **A. T. Valentine.**] Maximum values of osmotic concentration in plant tissue fluids.

1687. Weight and water content of leaf tissue in a tetracotyledenous mutant of phaseolus vulgaris.

Hart, Merrill C.

1618. See **Hirschfelder, Arthur D.**

Hastings, A. Baird.

1740. The lactic acid in the blood of dogs in exercise.

Hawk, Philip B.

1614. [with **Clarence A. Smith** and **Olaf Bergeim.**] The vitamine content of honey and honeycomb.

1634. [with **Clarence A. Smith** and **Olaf Bergeim.**] The nutritive value of extra-yeast bread.

1662. [with **Clarence A. Smith** and **Olaf Bergeim.**] The addition of yeast to a milk diet.

Heft, H. L.

1647. See **Eddy, Walter H.**

Hess, Alfred F.

1607. [with **Lester J. Unger** and **G. C. Supplee.**] The relation of fodder to the antiscorbutic potency and salt content of milk.

1650. [with **Lester J. Unger.**] The destructive effect of oxidation on antiscorbutic vitamine.

1717. [with **G. F. McCann** and **A. M. Pappenheimer.**] The failure of rats to develop rickets on a diet deficient in vitamine A.

1718. See **Pappenheimer, A. M.**

1735. [with **Lester J. Unger.**] The cure of infantile rickets by artificial light and by sunlight.

Hirschfelder, A. D.

1618. [with **Merrill C. Hart** and **Frank J. Kucera.**] Mercury saligenin, a new antiseptic.

Hofmann, W. F.

1632. See **Harris, J. Arthur.**

Howland, J.

1710. See **Tisdall, F. F.**

Hubbard, Roger S.

1741. [with **John A. Wentworth.**] A case of metastatic calcification associated with chronic nephritis and hyperplasia of the parathyroids.

Jackson, Henry Jr.

1642. [with **Walter W. Palmer.**] A modification of Folin's uric acid method.

Joffe, J. T.

1583. See **Waksman, S. A.**

Johnson, Ruth.

1647. See **Eddy, Walter H.**

Johnson, S. R.

1732. See **Kahn, R. L.**

Kahn, Max.

1681. [with **L. G. Hadjopoulos.**] A clinical method for the quantitative estimation of calcium in blood.

Kahn, R. L.

1663. The rate of fixation of complement at various temperatures.

1664. The quantitative relation between complement and complement fixing antibody.

1665. The thermostability of complement fixing antibodies resulting from protein immunization.

1731. [with **D. S. Lyon.**] Further studies on the affinity of sheep-corpuscles for anti-sheep hemolysin.

1732. [with **S. R. Johnson.**] The effect of heat on specific complement fixing antibodies.

1733. On the persistence of complement fixing antibodies in the serum of rabbits immunized with purified proteins.

Kipp, H. A.

1597. Variations in the total cholesterol content of the blood serum in pernicious anaemia and pneumonia.

Kleitman, Nathaniel.

1684. See **Salant, William.**

1708. See **Salant, William.**

1709. See **Salant, William.**

Knudson, Arthur.

1736. [with **Thomas Ordway** and **Hazel Ferguson.**] Cholesterol and cholesterol esters in blood showing a positive Wassermann reaction.

Kofoid, Charles A.

1743. [with **Olive Swezy.**] Councilmania lafleuri, a new amœba of the human intestine.

Kolmer, John A.

1654. See **Raiziss, George W.**

Kopeloff, Nicholas.

1742. [with **Sterne Morse.**] Studies on atmospheric requirements of bacteria: Water vapor tension.

Kramer, B.

1710. See **Tisdall, F. F.**

Krammer, M. M.
1607. See **Sherman, H. C.**

Kucera, Frank J.
1618. See **Hirschfelder, Arthur D.**

Kunkel, William M.
1613. See **Macht, D. I.**

Kuttner, Anne.
1610. See **Zinsser, Hans.**
1658. Preliminary report on a typhoid bacteriophage.
1694. On the influence of tissue enzymes on the bacteriophage principle.

Ladd, William S.
1633. [with **W. W. Palmer.**] The carbohydrate-fat ratio in relation to the production of ketone bodies in diabetes mellitus.

LaMar, Clifford L.
See **Stevens, Franklin A.**

LaMer, Victor K.
1602. [with **H. L. Campbell.**] Changes in organ weight produced by diets deficient in antiscorbutic vitamine.
1640. [with **H. L. Campbell** and **H. C. Sherman.**] The effect of temperature and of hydrogen ion concentration upon the rate of destruction of antiscorbutic vitamine.

Lange, Louis C.
1727. See **Barber, W. Howard.**

Leake, J. P.
1707. [with **J. N. Force.**] The inoculation of alastrim, or West Indian small-pox.

Levin, Isaac.
1734. See **Sturges, M. M.**

Levy, R. L.
1725. See **Cohn, A. E.**

Little, C. C.
1609. See **Strong, L. C.**
1635. [with **Marion Gibbons.**] Evidence for sex-linked lethal factors in man.

Loeb, Jacques.
1627. Chemical and molecular behavior of casein.

Loeb, Leo.

1656. The analysis of factors which determine the life and growth of transplanted tissues.

Lyon, D. S.

1731. See **Kahn, R. L.**

Macht, David L.

1598. [with **William Bloom.**] Effect of antipyretics on memory and behavior of albino rats.

1613. [with **William M. Kunkel.**] Concerning the antiseptic action of some aromatic fumes.

1619. [with **William Bloom.**] A pharmacodynamic analysis of cocain action of the cerebrum.

1628. [with **William Bloom.**] Comparative study of ethanol, caffein and nicotine on behavior of albino rats.

1629. [with **William Bloom.**] The effect of prostatectomy on the behavior and learning of albino rats.

1669. Pharmacological examination of cinnamein, benzyl succinate and benzyl nitrate.

1702. [with **William Bloom.**] Comparative study of ethanol, caffein and nicotine on the development of frog's larvæ.

MacDougal, D. T.

1621. The effects of yeast vitamine water-soluble B. on plant cell masses and on biocolloids.

1705. Effects of age and of the inclusion of salts on the heterotropic action of colloidal bodies of cytological interest.

Mackenzie, George M.

1690. [with **Louis B. Baldwin.**] Observations on the specific exhaustion of cutaneous reactions.

MacLeod, F. L.

1607. See **Sherman, H. C.**

MacNider, William DeB.

1617. On the elimination of phenolsulphonephthalein in acute mercuric chloride intoxication.

Mattill, H. A.

1703. The nutritive properties of milk with special reference to growth and reproduction in the white mouse.

Mavor, James W.

1737. On the elimination of the x-chromosome from the egg of drosophila melanogaster by x-rays.

McCann, G. F.

1717. See **Hesss, Alfred F.**

1718. See **Pappenheimer, A. M.**

McCluggage, H. B.

1648. See **Givens, M. H.**

1659. See **Givens, M. H.**

McCollum, E. V.

1721. [with **Nina Simmonds, P. G. Shipley** and **E. A. Park.**] Studies on experimental rickets: Cod liver oil as contrasted with butter fat in the protection against the effects of insufficient calcium in the diet.

1722. See **Shipley, P. G.**

McKee, Clara.

1667. See **Bull, Carroll G.**

Mendel, Lafayette B.

1645. See **Osborne, T. B.**

1661. See **Osborne, T. B.**

Moore, A. R.

1689. Chemical stimulation of the annelid nerve cord.

Morse, Sterne.

1742. See **Kopeloff, Nicholas.**

Morse, Withrow.

1706. Is glycogen the source of acids developed in autolysis?

Mueller, J. Howard.

1588. Observations on bacterial metabolism.

1695. Growth determining substances in bacteriological culture media.

Myers, Victor C.

1616. [with **James J. Short.**] The potassium content of normal and some pathological human bloods.

Olitsky, Peter K.

1624. See **Gates, F. L.**

Oliver, Jean.

1745. [with **Sosabro Yamada.**] The agglutinating action of salvarsan in vitro and in vivo.

Ordway, Thomas.
1736. See **Knudson, Arthur.**
Orr, P. F.
1671. See **Bronfenbrenner, J.**
Osborne, Thomas B.
1645. [with **Lafayette B. Mendel.**] Does growth require preformed carbohydrate in the diet?
1661. [with **Lafayette B. Mendel.**] Growth on diets containing more than ninety per cent. of protein.
Palmer, W. W.
1633. See **Ladd, W. S.**
1642. See **Jackson, Henry Jr.**
Pappenheimer, A. M.
1679. See **Sherman, H. C.**
1717. See **Hess, A. F.**
1718. [with **G. F. McCann, T. F. Zucker,** and **A. F. Hess.**] The effect of various modifications of a diet producing rickets in rats.
Park, E. A.
1721. See **McCollum, E. V.**
1722. See **Shipley, P. G.**
Parker, Julia T.
1610. See **Zinsser, Hans.**
1700. On the antigenic properties of ragweed pollen.
Patten, Mary W.
1675. The life history of an amicronucleate race of Didinium nasutum.
Pearce, Louise.
1682. See **Brown, Wade H.**
1683. See **Brown, Wade H.**
1713. See **Brown, Wade H.**
1714. [with **Wade H. Brown.**] Multiple infections with treponema pallidum in the rabbit.
Pellini, Emil J.
1636. See **Wallace, George B.**
Peters, John P. Jr.

1585. [with **David P. Barr.**] The carbon dioxid dissociation curve and the arterial and venous carbon dioxid tension of human blood in health and in disease.

Raiziss, George W.

1654. [with **Jay F. Schamberg** and **John A. Kolmer.**] Theoretical considerations bearing upon the chemotherapy of arsenical compounds.

Raymond, Edward.

1639. See **Zinsser, Hans.**

Reich, W. W.

1747. See **Beckwith, T. D.**

Rettger, Leo F.

1601. See **Cheplin, H. A.**

Rhodes, Bernice.

1603. See **Gay, F. P.**

Riddle, Oscar.

1622. Differential survival of male and female dove embryos in increased and decreased pressures of oxygen: A test of the metabolic theory of sex.

1631. General effects of increased and decreased pressures of oxygen on dove embryos.

Rohdenberg, G. L.

1600. See **Bullock, F. D.**

Rosenbloom, Jacob.

1698. A study of the Wassermann reaction in one hundred and forty cases of diabetes mellitus.

1699. Blood pressure studies in one hundred and forty cases of diabetes mellitus.

Salant, William.

1684. [with **Nathaniel Kleitman.**] The pharmacology of acetone.

1708. [with **Nathaniel Kleitman.**] Studies on the action of mercury.

1709. [with **Nathaniel Kleitman.**] The toxicity of skatol.

Sanger, B. J.

1637. The glucose mobilization rate in hyperthyroidism.

Schlesinger, M. J.

1625. See **Bronfenbrenner, J.**

1652. See **Bronfenbrenner, J.**
1671. See **Bronfenbrenner, J.**
1699. See **Bronfenbrenner, J.**
1712. See **Bronfenbrenner, J.**
1739. See **Bronfenbrenner, J.**

Schloss, Oscar M.
1630. A substance toxic to guinea pigs in the blood of infants with intestinal intoxication.

Schmidt, Carl L. A.
1685. The reaction of taurin with α-naphthylisocyanate.
1686. [with **G. L. Foster.**] A cheap and convenient source for glutamic acid.

Shamberg, J. F.
1654. See **Raiziss, George W.**

Sherman, H. C.
1607. [with **F. L. MacLeod** and **M. M. Kramer.**] Preliminary experiments with the fat-soluble vitamine.
1640. See **LaMer, V. K.**
1679. [with **A. M. Pappenheimer.**] A dietetic production of rickets in rats and its prevention by an inorganic salt.

Shevky, Richard.
1744. See **Dickson, Ernest C.**

Shipley, Paul G.
1721. See **McCollum E. V.**
1722. [with **E. A. Park, E. V. McCollum** and **Nina Simmonds.**] Studies on experimental rickets, II: The production of rickets by means of a diet faulty in only two respects.

Short, J. J.
1616. See **Myers, V. C.**

Simmonds, Nina.
1721. See **McCollum, E. V.**
1722. See **Shipley, P. G.**

Smith, Clarence A.
1614. See **Hawk, P. B.**
1634. See **Hawk, P. B.**
1662. See **Hawk, P. B.**

Spencer, Hope.
1672. See **Woodruff, L. L.**

1698. See **Woodruff, L. L.**
1701. See **Woodruff, L. L.**
1738. See **Woodruff, L. L.**

Starry, Allen C.
1620. See **Warthin, Aldred S.**

Stevens, Franklin A.
1697. See **West, Randolph.**
1716. [with **Clifford L. LaMar.**] Variations in the streptolysin curve in serum media.

Stevenson, H. C.
1647. See **Eddy, Walter H.**

Stone, Ruth L.
1604. The bactericidal action of rabbit bile on certain strains of streptococci.

Strong, L. C.
1609. [with **C. C. Little.**] Tests for physiological differences in transplantable tumors.

Sturges, M. M.
1734. [with **Isaac Levin.**] Influence of radium and x-rays on the frog's leucocytes.

Supplee, G. C.
1607. See **Hess, A. F.**

Swezy, Olive.
1743. See **Kofoid, Charles A.**

Thjotta, Th.
1680. [with **O. T. Avery.**] Growth accessory substances in the nutrition of bacteria.

Thro, William C.
1676. [with **Marie Ehn.**] Calcium in the blood.

Tisdall, F. F.
1710. [with **B. Kramer** and **J. Howland.**] The concentration of sodium and potassium compared with that of calcium and magnesium in the serum of patients with active infantile tetany.

Tokuda, Keiichi.
1655. Refactometric studies with the sera of syphilitic patients under arsphenamin and neo-arsphenamin treatment.

Tolstoi, E.
1673. See **Barbour, H. G.**
Uhlenhuth, Eduard.
1587. Experimental gigantism produced by feeding pituitary gland.
Unger, Lester J.
1607. See **Hess, Alfred F.**
1650. See **Hess, Alfred F.**
1735. See **Hess, Alfred F.**
Valentine, A. T.
1626. See **Harris, J. Arthur.**
1632. See **Harris, J. Arthur.**
Van Slyke, Donald D.
1649. [with **Carl A. L. Binger.**] The determination of lung volume without forced breathing.
Van Horne, E. G.
1648. See **Givens, M. H.**
Von Meysenbug, L.
1719. Diffusible calcium in normal, rachitic and experimental tetany blood.
Waksman, Selman A.
1583. [with **J. S. Joffre.**] The oxidation of sulfur by microorganisms.
1692. On the preparation of a soluble extract from soy beans.
Wallace, George B.
1636. [with **E. J. Pellini.**] Acidosis from capillary poisons.
Warthin, A. S.
1620. [with **Allen C. Starry.**] Improved methods for staining spirochæta pallida in tissue.
Weiss, Charles.
1688. [with **Anna Corson.**] Preliminary note on chemical changes in the blood of syphilitics under arsphenamin treatment.
Weiss, H.
1711. See **Bronfenbrenner, J.**

Wentworth, John A.
1741. See **Hubbard, Roger S.**
West, Randolph.
1697. [with **Franklin A. Stevens.**] The peptolytic enzymes of hemolytic streptococci; methods.
Wiggers, Carl J.
1651. The influence of venous return and arterial resistance on the pressures within the right and left ventricles.
Williams, H. B.
1670. New method for graphic study of heart murmurs.
Wilson, D. Wright.
1693. See **Bayne-Jones, S.**
Winkin, Cora S.
1657. The vasomotor response in anemia of the medulla oblongata: (1) The splanchnic vaso-constrictor fibres. (2) The relation of the splanchnic constrictor fibres to the secretion of adrenalin.
Woodruff, L. L.
1599. Amicronucleate infusoria.
1646. *Paramecium calkinsi* sp. n.
1672. [with **Hope Spencer.**] The food reactions of the infusorian *Spathidium spathula.*
1701. [with **Hope Spencer.**] The early effects of conjugation on the division rate of *Spathidium spathula.*
1738. [with **Hope Spencer.**] The survival value of conjugation in the life history of *Spathidium spathula.*
Wu, S. T.
1715. See **Zinsser, Hans.**
Yamada, Sosabro.
1745. See **Oliver, Jean.**
Zinsser, Hans.
1610. [with **Julia T. Parker** and **Ann Kuttner.**] On certain poisonous substances produced in bacterial cultures.
1611. Observations on anaphylaxis in lower monkeys.
1639. [with **Edward H. Raymond, Jr.**] On a method of producing chronic focal lesions in animals.
1641. The tuberculin reaction and anaphylaxis as studied by the Dale method.

1715. [with **S. T. Wu.**] Report on anaphylactic deaths in guinea pigs from intracutaneous injection of small amounts of egg albumin.

Zucker, Theodore F.

1718. See **Pappenheimer, A. M.**

1720. The relation of acid base equilibrium in the body to excretion of phosphorus and calcium.

EXECUTIVE PROCEEDINGS.

Main Society.

One Hundred Ninth Meeting.

Cornell University Medical College, October 20, 1920. *President Calkins in the chair.*

Members present: Auer, Burton-Opitz, Calkins, Churchman, Dubois, Edwards, D. J., Goldschmidt, Hess, Jackson, H. C., Lusk, Moore, Myers, Olitsky, Pappenheimer, Rose, A. R., Torrey, Uhlenhuth, Waksman.

Members elected: Harry L. Alexander, David P. Barr, Norman R. Blatherwick, John Francis Cowan, Lawrence J. Henderson, Benjamin Kramer, Lovell Langstroth, William S. McCann, Frank Maltaner, J. Howard Mueller, John P. Peters Jr., Edward C. Schneider, Edward B. Towne, D. Wright Wilson.

Honorary members elected: Edward T. Reichert.

One Hundred Tenth Meeting.

New York Post-Graduate Medical School, November 17, 1920. *President Calkins in the chair.*

Members present: Bailey, Baehr, Calkins, Chambers, Coleman, Eddy, Funk, Gies, Greenwald, Jackson, H. C., Jobling, Kleiner, Little, MacNeal, Maltaner, McCann, Mueller, Myers, Olitsky, Pappenheimer, Rose, A. R., Sherman, Sherwin, Sittenfield, Stark, Thro, Waksman, Wallace.

Members elected: Burrill Crohn, Paul De Kruif, Robert H. Halsey, Julia T. Parker.

One Hundred Eleventh Meeting.

Rockefeller Institute for Medical Research, December 15, 1920. *President Calkins in the chair.*

Members present: Auer, Baehr, Burton-Opitz, Calkins, Churchman, Eddy, Funk, Gates, Goldfarb, Harris, Hastings, Hess, Jackson, H. C., Kleiner, Kober, Loeb, Mueller, Olitsky, Oppenheimer, Ottenberg, Pike, Rose, A. R., Scott, Stark, Thomas, Thro, Uhlenhuth, Waksman, Wallace, Zingher.

Members elected: H. E. Dubin, P. D. Lamson, Theodore F. Zucker.

Meltzer Memorial Meeting.

Academy of Medicine, January 6, 1921. *President Calkins in the chair.*

This meeting was held in conjunction with the Academy of Medicine and the Harvey Society.

The following members of the Society spoke: Gary N. Calkins, William H. Howell, Phoebus A. Levene, Graham Lusk, George B. Wallace, and William H. Welch.

These papers are published as a memorial number of the Proceedings.

One Hundred Twelfth Meeting.

College of Physicians and Surgeons, January 19, 1921. *President Calkins in the chair.*

Members present: Austin, Bailey, Burton-Opitz, Calkins, Chambers, Clowes, Coombs, Eddy, Edwards, Eggstein, Goldfarb, Goldschmidt, Harris, Hasting, Hess, Jackson, H. C., Jobling, Killian, Lieb, Little, Macht, Myers, Parker, Peters, Pike, Riddle, Rose, Rosenbloom, Schloss, Sherman, Stark, Teague, Thro, Van Slyke, Wallace, Zingher, Zinsser, Zucker.

Members elected: Louise H. Gregory, Clara J. Lynch, Ralph G. Stillman.

Nominations for the ensuing year were made as follows: President,—Wallace, Vice-President,—Jobling, Secretary-Treasurer,—Jackson, H. C., Members of the Council,—Myers, V. C. Van Slyke, Goldfarb.

One Hundred Thirteenth Meeting. (Eighteenth Annual Meeting.)

College of the City of New York, February 16, 1921. *President Calkins in the chair.*

Members present: Auer, Bailey, C. V., Blakeslee, Browne, W. W., Calkins, Cohen, Coombs, Crampton, Dubin, Eddy, Edwards, Fine, Goldfarb, Gregory, Halsey, Hess, Jackson, H. C., Jobling, Kahn, M., Killian, Kleiner, Lusk, Lynch, MacNeal, Myers, Pike, Raiziss, Riddle, Rose, Rosenbloom, Scott, G. Senior, Sherman, Stark, Stillman, Teague, Thro, Uhlenhuth, Wallace, Weiss, Wollstein, Zingher.

Members elected: T. D. Beckwith, William J. Crozier, Ivan C. Hall, Robert L. Levy, Baldwin Lucké, D. E. Lancefield, Eli Kennerly Marshall, Ernest Gale Martin, Emil J. Pellini, Julius M. Rogoff.

Resignations: David C. Edsall.

Officers of the Society elected: George B. Wallace, President, James W. Jobling, Vice-President, Holmes C. Jackson, Secretary-Treasurer, Member of the Council, Victor C. Myers.

The annual dinner was held at the College of the City of New York following the one hundred thirteenth meeting. The following members were present: Auer, Bailey, Baitsell, Blakeslee, Cohen, Coombs, Crampton, Calkins, Dubin, Eddy, Edward, Fine, Famulener, Gies, Goldfarb, Harris, Halsey, Hess, Jackson, H. C., Jobling, Kahn, M., Killian, Kleiner, Lusk, Marine, Metz, Myers, MacDonell, MacNeal, Peters, Pike, Pease, Pappenheimer, Raiziss, Riddle, Rose, Rosenbloom, Scott, Senior, Sherwin, Stillman, Uhlenhuth, Thro, Wallace, Weiss, Zingher.

Dr. Gies and Dr. Lusk were appointed a committee to prepare memorial resolutions on the death of Dr. Meltzer to be included in the memorial number of the PROCEEDINGS.

One Hundred Fourteenth Meeting.

College of Physicians and Surgeons, March 16, 1921. *President Wallace in the chair.*

Members present: Auer, Austin, Bailey, C. V., Baumann, Brown, Cohn, Cole, R., Dubin, Eddy, Eggstein, Gies, Greenwald, Halsey, Harris, Hess, Jackson, H. C., Jobling, Killian, Lieb, Longcope, Lusk, MacNeal, Myers, Oppenheimer, Pappenheimer, Parker, Peters, Robinson, Rose, A. R., Sherman, Stark, Swift, Thro, Wallace, Williams, Watanabe, Zucker.

Members elected: Carl A. L. Binger, Roger Sanford Hubbard, James P. Leake, George M. Mackenzie, Thurlow C. Nelson, Franklin A. Stevens, Karl M. Vogel.

Announcement was made by the Secretary of a gift of $2000 from the Meltzer family to be applied to an endowment for the Society, known as the Meltzer Memorial Fund. The Society passed a vote of thanks which was forwarded by the Secretary.

The Meltzer Memorial resolution was read and passed.

One Hundred Fifteenth Meeting.

University and Bellevue Hospital Medical College, April 20, 1921. *President Wallace in the chair.*

Members present: Binger, Burton-Opitz, Calkins, Coca, Eddy, Fine, Funk, Gies, Goldschmidt, Greenwald, Hooper, Jackson, H. C., Jobling, Killian, Mackenzie, MacNeal, McCann, Moore, Myers, Mueller, Pappenheimer, Parker, Pellini, Peters, Rosenbloom, Senior, Stevens, Teague, Waksman, Wallace, Wilson, Zucker.

Members elected: O. T. Avery, E. T. Bell, E. D. Brown, Maynie R. Curtis, R. A. Dutcher, C. H. Eckles, C. P. Fitch, H. K. Hayes, A. T. Henrici, E. C. Kendall, F. B. Kingsbury, W. P. Larson, Michael Levine, E. J. Lund, F. C. Mann, William Moore, L. S. Palmer, H. E. Robertson, F. H. Scott, E. C. Stackman, J. J. Willaman.

Resignations: Walter E. Dandy, Walter Jones.

A request was granted for the founding of a Minnesota branch of the Society.

One Hundred Sixteenth Meeting.

Columbia University, May 18, 1921. *President Wallace in the chair.*

Members present: Auer, Austin, Binger, Calkins, Dubin, Eddy, Funk, Gies, Gregory, Harris, I., Hastings, Hess, Jackson, H. C., Jobling, Knudson, Levy, Marine, Myers, V. C., Oppenheimer, Pappenheimer, Park, Peters, Rose, A. R., Sherman, Sittenfield, Uhlenhuth, Wallace, Zucker.

Members elected: Glen E. Cullen, Robert S. Cunningham, Florence Hulton Frankel, H. F. Höst, Morris Hirsch Kahn, E. V. McCollum, William C. Rose, Paul G. Shipley, R. E. Scammon.

Resignations: F. J. Birchard.

The following amendments to the constitution were voted upon and passed:

That Art. IV. Sec. 2, Annual Business, which now reads "The first regular meeting of each calendar year shall be an annual business meeting," shall be amended to read—"*The April meeting shall be the annual business meeting.*"

That Art. V. Sec. 4, Term of Office, which now reads, "The term of office shall be one calendar year except as specified above for the Council," shall be amended to read—"*The term of office shall be one academic year beginning with the October meeting, except as specified for members of the Council elected at large from the Society.*" This exception for members of the Council is given in Art. V. Sec. 2, Council, which reads—"The Council shall consist of the President, the Vice-president, the Secretary-treasurer and two members of the Society, one elected annually to serve two years."

That the following sections be added to Article III—Membership. Section 2—*Forfeiture.*

(B) Any member who conducts an investigation which, in the opinion of the Council, involves unnecessary suffering or inflicts injury upon any person without the understanding consent of that person or that person's guardian, shall forfeit his membership.

(C) Any member who, in the opinion of the Council, conducts an investigation that involves unnecessary suffering by animals, shall forfeit his membership.

Amendments to the By-Laws:

V. *Procedure for the determination of guilt under charges affecting tenure of membership.*

(A) Any member who may be charged formally and in writing with conduct rendering him subject to the constitutional provisions of forfeiture of, or expulsion from, membership, shall be given ample opportunity to show to the Council that the said charges are unfounded.

(B) If, after a full hearing, the Council finds, by a majority vote of its total membership, that a member is guilty of conduct rendering him subject to the constitutional provisions relative to forfeiture of, or expulsion from, membership, the Council shall, in the first instance, promptly make due formal announcement of such forfeiture; or, in the second instance, shall formally propose the expulsion of that member, with a statement of the reasons therefor, by a mode of procedure in accord with the general provisions of Art. III, Sec. 4 of the Consti-

tution, but in a manner that will adequately afford the member involved every privilege of appeal to all the members of the Society against the proposed expulsion.

PACIFIC COAST BRANCH.

Twenty-Sixth Meeting.

University of California, Berkeley, October 13, 1920.

Members present: Addis, Barnett, Burnett, Bloor, Dixon, Evans, Faber, Gay, Hewlett, Langstroth, Mehrtens, Oliver, Ophüls, Schmidt, Smith, Towne, Whipple.

Twenty-Seventh Meeting.

Stanford Medical School, San Francisco, January 12, 1921.

Members present: Barnett, Bloor, Cowan, Dickson, Evans, Faber, Fleischner, Hewlett, Langstroth, Lucas, Oliver, Ophüls, Schmidt, Smith, Walker, Whipple.

Twenty-Eight Meeting.

University of California Hospital, San Francisco, March 9, 1921.

Members present: Addis, Barnett, Beckwith, Bloor, Dickson, Evans, Faber, Langstroth, Lucas, Martin, Morgan, T.H., Schmidt, Walker, Whipple.

Twenty-Ninth Meeting.

University of California, Berkeley, May 4, 1921.

Members present: Beckwith, Blatherwick, Bloor, Dickson, Evans, Gay, Hall, Kofoid, Lucas, Martin, Oliver, Ophüls, Schmidt, Smith, Walker.

REGISTER OF NAMES AND INSTITUTIONAL CONNECTION OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

HONORARY MEMBERS.

Councilman, William T.	Harvard University
Reichert, Edward T.	University of Pennsylvania

ACTIVE MEMBERS.

Abbott, Alexander C.	University of Pennsylvania
Abel, John F.	Johns Hopkins University
Adami, J. George	University of Liverpool, England
Addis, Thomas	Lane Hospital, San Francisco
Adler, Herman M.	Juvenile Psychopathic Institute, Chicago
Alexander, Harry L.	Cornell University Medical College, N. Y. City
Allen, Bennet M.	University of Kansas
Alsberg, Carl L.	U. S. Department of Agriculture, Washington, D. C.
Alvarez, Walter C.	University of California, Medical School
Amoss, Harold L.	Rockefeller Institute, N. Y. City
Anderson, John F.	Rutgers College
Atkinson, James P.	New York City Health Department
Auer, John	Rockefeller Institute, N. Y. City
Austin, J. Harold	Rockefeller Institute, N. Y. City
Avery, O. T.	Rockefeller Institute, N. Y. City
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bailey, C. H.	Columbia University
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Bailey, Harold C.	Cornell University Medical College, N. Y. City
Baitsell, George A.	Yale University
Balls, A. K.	Peekskill, New York
Banta, A. M.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Banzhaf, Edwin J.	N. Y. Health Department
Barbor, W. Howard	New York University
Barbour, Henry G.	Yale University
Bardeen, Charles R.	University of Wisconsin
Barnett, George D.	Leland Stanford University
Barr, David P.	Cornell University Medical College, N. Y. City
Bauman, Louis	Presbyterian Hospital, N. Y. City
Beckwith, T. D.	University of California
Bell, E. T.	University of Minnesota
Benedict, S. R.	Cornell University Medical College, N. Y. City
Berg, William N.	Bureau of Animal Industry, Washington, D. C.
Bergeim, Olaf	Jefferson Medical College

Bergey, David H.	University of Pennsylvania
Binger, Carl A. L.	Hospital of the Rockefeller Institute
Blakesley, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.	Memorial Laboratory and Clinic, Santa Barbara
Bloor, W. R.	University of California
Boeck, William C.	Hygienic Laboratory, Washington, D. C.
Bronfenbrenner, J.	Harvard Medical School
Brooks, Harlow	New York University
Brooks, S. C.	Hygienic Laboratory, Washington, D. C.
Brown, Wade H.	Rockefeller Institute, N. Y. City
Browne, W. W.	College of the City of New York
Bull, C. G.	Johns Hopkins University
Bunting, C. H.	University of Wisconsin
Burnett, Theodore C.	University of California
Burrows, M. T.	Washington University Medical School
Burton-Opitz, Russell	Columbia University

Calkins, Gary N.	Columbia University
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carrel, Alexis	Rockefeller Institute, N. Y. City
Caulfeild, A. H.	Toronto, Canada
Cecil, R. L.	Bellevue Hospital, N. Y. City
Chace, Arthur F.	N. Y. Post-Graduate Medical School
Chambers, Robert	Cornell University Medical College, N. Y. City
Chidester, F. E.	University of West Virginia
Chittenden, R. H.	Yale University
Churchman, John W.	N. Y. City
Clark, P. F.	University of Wisconsin
Clowes, G. H. A.	Indianapolis, Indiana
Coca, A. F.	Cornell University Medical College, N. Y. City
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohen, Barnett	U. S. Hygienic Laboratory, Washington, D. C.
Cohen, Martin	N. Y. Post-Graduate Medical School
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Coleman, Warren	New York University
Collins, Katharine R.	Division of Laboratories, Buffalo City Hospitals
Conklin, E. G.	Princeton University
Cooke, J. V.	Washington University Medical School
Coombs, Helen C.	Columbia University
Corner, George V.	Johns Hopkins Medical School
Cort, W. W.	Johns Hopkins University
Cowan, John F.	Stanford University Hospital, San Francisco
Crampton, C. Ward	Department of Education, New York City
Crile, George W.	Western Reserve University
Crohn, Burrill	Mt. Sinai Hospital, N. Y. City
Crozier, W. J.	Rutgers College
Cunningham, R. S.	Johns Hopkins University

Curtis, Maynie R.	Columbia University
Cushing, Harvey W.	Harvard Medical School

Dakin, H. D.	Ossining, N. Y.
Davenport, C. B.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
DeKruif, Paul H.	Rockefeller Institute, N. Y. City
Dickson, E. C.	Stanford University Medical School
Dochez, A. A.	Johns Hopkins University
Donaldson, H. H.	Wister Institute, Philadelphia
Draper, George W.	Columbia University
Draper, John W.	New York City
Dresbach, M.	Albany Medical College
Dubin, Harry E.	Industrial Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College, N. Y. City
Duggar, B. M.	Missouri Botanical Garden
Dunham, E. K.	N. Y. University
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University

Eckles, C. H.	University of Minnesota
Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College, N. Y. City
Eggleston, Cary	Cornell University Medical College, N. Y. City
Eggstein, Andrew	Columbia University
Eisenbrey, A. B.	Western Reserve University
Elsberg, Charles A.	Mt. Sinai Hospital, N. Y. City
Elser, W. J.	Cornell University Medical College, N. Y. City
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda	Berlin, Germany
Erlanger, Joseph	Washington University Medical School
Evans, Herbert M.	University of California
Ewing, E. M.	Asheville, North Carolina
Ewing, James	Cornell University Medical College, N. Y. City
Eyster, J. A. E.	University of Wisconsin

Faber, Harold K.	Stanford Medical School, San Francisco
Fahr, George	Montefiore Hospital, N. Y. City
Falk, K. George	Roosevelt Hospital, N. Y. City
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Field, Cyrus W.	New York City
Fine, M. S.	Newark, New Jersey
Fischer, Martin H.	General Hospital, Cincinnati
Fitch, C. P.	University of Minnesota, St. Paul
Fitzgerald, J. G.	University of Toronto
Fleischner, E. C.	University of California, San Francisco
Flexner, Simon	Rockefeller Institute, N. Y. City
Flournoy, Thomas	House of Mercy Hospital, Pittsfield, Mass.
Folin, Otto	Harvard Medical School
Ford, W. W.	Johns Hopkins University

Foster, Nellis B.	New York Hospital, N. Y. City
Frankel, Florence Hulton	New York University
Funk, Casimir	N. Y. City
Gager, C. Stuart	Brooklyn Botanical Gardens
Gates, Frederick L.	Rockefeller Institute, N. Y. City
Gay, F. P.	University of California
Gaylord, H. R.	Gratwick Laboratory, Buffalo
Gesell, Robert A.	University of California
Gettler, A. O.	New York University
Gies, William J.	Columbia University
Githens, T. S	Philadelphia, Penn.
Givens, Maurice H.	Western Pennsylvania Hospital, Pittsburgh
Glaser, Otto	Amherst College.
Goldfarb, A. J.	College of the City of New York
Goldschmidt, Samuel	Cornell University Medical College, N. Y. City
Gortner, R. A.	University of Minnesota, St. Paul
Greenwald, Isidor	Roosevelt Hospital, N. Y. City
Gregory, Louise H.	Barnard College
Guenther, A. E.	University of Nebraska
Guthrie, C. C.	University of Pittsburgh

Hale, Worth	Harvard Medical School
Hall, Ivan C.	University of California
Halsey, Robert	N. Y. Post-Graduate Medical School
Halsted, W. S.	Johns Hopkins University
Hanzlik, P. J.	Western Reserve Medical College
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, J. Arthur	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Harrison, R. G.	Yale University
Hartwell, John A.	Cornell University Medical College, N. Y. City
Harvey, E. Newton	Princeton University
Hastings, A. Baird	Columbia University
Hatai, Shinkishi	Tohoku Imperial University
Hatcher, R. A.	Cornell University Medical College, N. Y. City
Hawk, P. B.	Jefferson Medical College
Hayes, H. K.	University of Minnesota, St. Paul
Hegner, R. W.	Johns Hopkins University
Henderson, Lawrence J.	Harvard Medical School
Hendrix, B. M.	University of Pennsylvania
Henrici, Arthur T.	University of Minnesota
Hess, Alfred F.	New York University
Hewlett, A. W.	Stanford University Medical School
Hirschfelder, Arthur	University of Minnesota
Holman, W. L.	Leland Stanford University
Holmes, S. J.	University of California
Hooker, Davenport	University of Pittsburgh
Hooper, Charles W.	Brooklyn, N. Y.
Hopkins, J. Gardner	Columbia University
Hoskins, R. G.	Johns Hopkins University
Howe, Paul E.	Rockefeller Institute, N. Y. City

Howell, William H.	Johns Hopkins University
Howland, John	Johns Hopkins Hospital, Baltimore
Huber, G. Carl	University of Michigan
Hubbard, Roger S.	Clifton Springs Sanitarium, N. Y.
Hunt, Reid	Harvard Medical School
Hunter, Andrew	University of Toronto
Hurwitz, Samuel	University of California, San Francisco
Jackson, D. E.	University of Cincinnati
Jackson, Holmes C.	New York University
Jacobs, Walter A.	Rockefeller Institute, N. Y. City
Janney, Nelson W.	Los Angeles, Cal.
Jennings, H. S.	Johns Hopkins University
Jobling, J. W.	Columbia University
Jones, Frederick S.	Rockefeller Institute, N. Y. City
Jordan, H. E.	University of Virginia
Joseph, Don R.	St. Louis University Medical School
Kahn, Max	Beth Israel Hospital, N. Y. City
Kahn, R. L.	Michigan Department of Health, Lansing
Karsner, H. T.	Lakeside Hospital, Cleveland
Kast, Ludwig	N. Y. Post-Graduate Medical School
Kellogg, V. L.	National Research Council, Washington, D. C.
Kendall, E. C.	University of Minnesota, Rochester
Killian, J. A.	N. Y. Post-Graduate Medical School
Kingsbury, F. B.	University of Minnesota
Kinsella, Ralph A.	St. Louis University Medical School
Kirkbride, Mary B.	Hygienic Laboratories, Albany, N. Y.
Kleiner, I. S.	Flower Hospital, N. Y. City
Kligler, I. J.	Rockefeller Institute, N. Y. City
Kline, B. S.	Montefiore Home, N. Y. City
Klotz, Oskar	University of Pittsburgh
Knudson, Arthur	Albany Medical College
Kober, Phillip A.	New Brunswick, N. J.
Kocher, R. A.	Trudeau Sanatorium, Saranac Lake, N. Y.
Kofoid, Charles A.	University of California
Kolmer, John A.	University of Pennsylvania
Kramer, Benjamin	Johns Hopkins Hospital, Baltimore, Md.
Krumbhaar, E. B.	Philadelphia General Hospital, Philadelphia
Lamar, R. V.	University of Georgia
Lambert, R. A.	Yale University
Lamson, Paul D.	Johns Hopkins University
Lancefield, D. E.	Columbia University
Langstroth, Lovell	University of California, San Francisco
Larson, W. P.	University of Minnesota
Laughlin, H. H.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry	Yale University
Leake, J. P.	Hygienic Laboratory, Washington, D. C.

LEATHES, J. B.	University of Sheffield, England
LEE, FREDERICK S.	Columbia University
LEVENE, P. A.	Rockefeller Institute, N. Y. City
LEVIN, ISAAC	New York University
LEVINE, MICHAEL	Montefiore Hospital, N. Y. City
LEVY, ROBERT L.	Rockefeller Institute, N. Y. City
LEWIS, HOWARD B.	University of Illinois
LEWIS, PAUL A.	Phipps Institute, Philadelphia
LIEB, C. C.	Columbia University
LILLIE, FRANK R.	University of Chicago
LILLIE, RALPH S.	Nela Research Laboratory, Cleveland
LITTLE, C. C.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
LOEB, JACQUES	Rockefeller Institute, N. Y. City
LOEB, LEO	Washington University Medical College
LOEVENHART, A. S.	University of Wisconsin
LOMBARD, WARREN P.	University of Michigan
LONGCOPE, W. T.	Columbia University
LUCAS, WILLIAM P.	University of California Hospital, San Francisco
LUCKÉ, BALDWIN	University of Pennsylvania
LUCKHARDT, A. B.	University of Chicago
LUND, E. J.	University of Minnesota
LUNDSGAARD, CHRISTEN	University of Copenhagen
LUSK, GRAHAM	Cornell University Medical College, N. Y. City
LYLE, W. G.	Roosevelt Hospital, N. Y. City
LYNCH, CLARA J.	Rockefeller Institute, N. Y. City
LYON, E. P.	University of Minnesota
MACALLUM, A. B.	Magill University, Montreal
MACCALLUM, W. G.	Johns Hopkins Hospital, Baltimore
MACDOUGAL, D. T.	Desert Laboratory, Tucson, Arizona
MACDOWELL, E. CARLTON	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
MACKENZIE, GEORGE M.	Presbyterian Hospital, N. Y. City
MACLEOD, J. J. R.	University of Toronto
MACNEAL, WARD J.	N. Y. Post-Graduate Medical School
MACNIDER, WILLIAM DEB.	University of North Carolina
MACCANN, WILLIAM S.	Cornell University Medical College, N. Y. City
MACHT, DAVID I.	Johns Hopkins University
MCCOLLUM, E. V.	Johns Hopkins University
MCCRUDDEN, FRANCIS M.	Robert Brigham Hospital, Boston
MCLEAN, FRANKLIN C.	Peking Union Medical College, China
MCMEANS, J. W.	University of Pittsburgh
MALTANER, FRANK	N. Y. State Department of Health, Albany
MANDEL, ARTHUR R.	New York University
MANDEL, JOHN A.	New York University
MANN, FRANK C.	University of Minnesota
MANWARING, W. H.	Leland Stanford University
MARINE, DAVID	Montefiore Home and Hospital, N. Y.
MARSHALL, E. K., JR.	Washington University Medical School
MARTIN, E. G.	Leland Stanford University

Maxwell, S. S.	University of California
Mayer, A. G.	Carnegie Institute, Washington, D. C.
Merhtens, Henry G.	Stanford University Hospital, San Francisco
Meigs, E. B.	U. S. Bureau of Animal Industry, Washington, D. C.
Mendel, Lafayette B.	Yale University
Metz, Charles W.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Meyer, Adolf	Johns Hopkins Hospital, Baltimore
Meyer, A. L.	Johns Hopkins University
Meyer, G. M.	Rockefeller Institute, New York City
Meyer, K. F.	University of California
Mohler, J. R.	U. S. Bureau of Animal Industry, Washington, D. C.
Moore, A. R.	Rutgers College
Moore, William	University of Minnesota
Morgan, T. H.	Columbia University
Morse, Withrow	University of West Virginia
Mossenthal, H. O.	N. Y. Post-Graduate Medical School
Mueller, J. Howard	Columbia University
Muller, Herman J.	University of Texas
Murlin, John R.	University of Rochester
Murphy, J. B.	Rockefeller Institute, N. Y. City
Musser, John H.	University of Pennsylvania
Myers, Victor C.	N. Y. Post-Graduate Medical School

Nelson, Thurlow C.	Rutgers College
Niles, Walter L.	Cornell University Medical College, N. Y. City
Noble, H. C.	New York University
Noguchi, H.	Rockefeller Institute, N. Y. City
Norris, Charles	Chief Medical Examiner, N. Y. City
Northrup, John H.	Rockefeller Institute, N. Y. City
Novy, Frederick G.	University of Michigan

Oertel, Horst	Magill University, Canada
Olitsky, Peter K.	Rockefeller Institute, N. Y. City
Oliver, Jean	Stanford University Medical School
Ophüls, William	Stanford University Medical School
Opie, Eugene L.	Washington University Medical School
Oppenheimer, B. S.	Columbia University
Osborne, Thomas B.	Agricultural Exp. Station, New Haven, Conn.
Osterhaut, W. J. V.	Harvard Medical School
Ottenberg, R.	Mt. Sinai Hospital, N. Y. City

Palmer, Leroy S.	University of Minnesota, St. Paul
Palmer, W. W.	Presbyterian Hospital, New York City
Pappenheimer, A. M.	Columbia University
Park, E. A.	Johns Hopkins Hospital, Baltimore
Park, William H.	Department of Health, N. Y. City
Parker, George H.	Harvard University
Parker, Julia T.	Columbia University
Peabody, Francis W.	Peter Bent Brigham Hospital, Boston
Pearce, Louise	Rockefeller Institute, N. Y. City

Pearl, Raymond	Johns Hopkins University
Pease, Marshall C.	N. Y. Post-Graduate Medical School
Pellini, Emil J.	New York University
Pemberton, Ralph	Presbyterian Hospital, Philadelphia
Pepper, O. H. Perry	University of Pennsylvania
Peters, John P., Jr.	Rockefeller Institute, N. Y. City
Petersen, W. F.	Chicago, Illinois
Pfaff, Franz	Harvard University
Pfeifer, J. A. F.	Baltimore, Maryland
Pike, F. H.	Columbia University
Plotz, Harry	Mt. Sinai Hospital, N. Y. City
Porter, William T.	Harvard University
Pratt, Joseph H.	Harvard University
Prince, A. L.	Wethersfield, Conn.

Raiziss, George W.	Dermatological Research Laboratories, Philadelphia
Ravenel, M. P.	University of Missouri
Retger, L. F.	Yale University
Richards, Alfred N.	University of Pennsylvania
Richards, Herbert M.	Columbia University
Riddle, Oscar	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Ringer, A. I.	New York City
Robertson, H. E.	University of Minnesota
Robertson, T. B.	University of Adelaide, South Australia
Robinson, G. Canby	Johns Hopkins University
Rogoff, J. M.	Western Reserve Medical School
Rose, Anton R.	N. Y. Post-Graduate Medical School
Rose, Mary Schwartz	Columbia University
Rose, William C.	University of Texas
Rosenau, M. J.	Harvard Medical School
Rosenbloom, Jacob	New York City
Roth, George B.	U. S. Department of Health, Washington, D. C.
Rothschild, M. A.	Mt. Sinai Hospital, N. Y. City
Rous, Payton	Rockefeller Institute, N. Y. City
Ryan, A. H.	Waterbury, Conn.

Salant, William	University of Georgia
Scammon, R. E.	University of Minnesota
Schloss, Oscar M.	Cornell University Medical College
Schlutz, F. W.	University of Minnesota
Schmidt, Carl L. A.	University of California
Schneider, Edward C.	Wesleyan University
Schultz, W. H.	George Washington University
Schwyzer, Fritz	Kastanienbaum, Switzerland
Scott, E. L.	Columbia University
Scott, F. H.	University of Minnesota
Scott, G. G.	College of the City of New York
Scott, R. W.	Western Reserve Medical College
Senior, Harold D.	New York University
Shaffer, Phillip A.	Washington University Medical School

Shaklee, A. O.	University of Texas
Sherman, H. C.	Columbia University
Sherwin, Carl P.	Fordham University
Shipley, Paul G.	Johns Hopkins University
Shive, J. W.	N. J. State Agr. Exp. Station, New Brunswick
Siler, J. F.	Office of the Surgeon General, Washington
Simon, C. E.	University of Maryland
Simpson, Sutherland	Cornell University
Sittenfield, M. J.	Columbia University
Smith, Arthur H.	Yale University
Smith, Phillip E.	University of California
Smith, Theobald	Rockefeller Institute, Princeton
Sollman, Torald	Western Reserve University
Spaeth, R. A.	Johns Hopkins University
Spoehr, H. A.	Desert Laboratory, Tucson, Arizona
Stakman, E. C.	University of Minnesota
Stark, Mary B.	Flower Hospital, N. Y. City
Stevens, Franklin A.	Presbyterian Hospital, N. Y. City
Stewart, G. N.	Western Reserve Medical School
Stiles, Percy G.	Harvard Medical School
Stillman, Ralph G.	New York Hospital, N. Y. City
Stockard, Charles R.	Cornell University Medical College
Stookey, Lyman B.	University of Southern California
Storey, Thomas A.,	U. S. Interdepartmental Social Hygiene Board, Washington, D. C.
Strong, Richard P.	Harvard Medical School
Strouse, Solomon	Northwestern University
Sturtevant, A. H.	Columbia University
Swain, R. E.	Leland Stanford University
Sweet, J. Edmund	University of Pennsylvania
Swift, H. F.	Columbia University
Symmers, Douglas	New York University, N. Y. City
Taliaferro, W. H.	Johns Hopkins University
Tashiro, Shiro	University of Cincinnati
Taylor, Allonzo E.	University of Pennsylvania
Taylor, R. M.	Maceo, Kentucky
Teague, Oscar	Columbia University
Ten-Broeck, Carl	Peking Union Medical College, China
Terry, B. T.	Vanderbilt School of Medicine
Thomas, Arthur W.	Columbia University
Thro, William C.	Cornell University Medical College, N. Y. City
Todd, John L.	Magill University, Montreal
Torrey, John C.	Cornell University Medical College, N. Y. City
Towne, Edward B.	Lane Hospital, San Francisco
Tyzzer, E. E.	Harvard Medical School
Uhlenhuth, Eduard	Rockefeller Institute, N. Y. City
Underhill, Frank P.	Yale University

Van Slyke, Donald D.	Rockefeller Institute, N. Y. City
Vogel, Karl M.	Columbia University

Wadsworth, Augustus B.	N. Y. State Department of Health, Albany
Waksman, S. A.	N. J. State Agr. Experiment Station, New Brunswick, N. J.
Walker, E. L.	University of California, San Francisco
Wallace, George B.	New York University
Warthin, Aldred S.	University of Michigan
Wasteneys, H.	University of Toronto, Canada
Watanabe, C. K.	Tokyo, Japan
Weiss, Charles	University of Pennsylvania
Welch, William H.	Johns Hopkins University
Welker, W. H.	University of Illinois
Weller, Carl V.	University of Michigan
West, C. J.	National Research Council, Washington, D. C.
Whipple, George H.	University of California, San Francisco
White, Benjamin	Antitoxin and Vaccine Laboratory, Boston
White, O. E.	Brooklyn Botanical Garden, Brooklyn, N. Y.
Wiggers, Carl J.	Western Reserve University
Willaman, J. J.	University of Minnesota, St. Paul
Williams, Anna W.	Department of Health, N. Y. City
Williams, Horatio B.	Columbia University
Williams, H. U.	University of Buffalo
Wilson, D. Wright	Johns Hopkins University
Wilson, Edmund B.	Columbia University
Winslow, C. E. A.	Yale University
Wolbach, S. B.	Harvard University
Wolf, C. G. L.	Addenbrooke's Hospital, Cambridge, England
Wolstein, Martha	Rockefeller Institute, N. Y. City
Wood, Francis C.	Columbia University
Woodruff, L. L.	Yale University

Yatsu, Naohide	Keo University, Tokyo, Japan
Yerkes, Robert M.	Washington, D. C.

Zingher, Abraham	Department of Health, N. Y. City
Zinsser, Hans	Columbia University
Zucker, Theodore	Columbia University

Total number of members at the close of the academic year, 1920–21:–448.

OFFICERS.

1903–1920.

	1903–'04	1904–'05	1905–'06	1906–'07	1907–'08	1908–'09
President	Meltzer	Meltzer	Wilson	Flexner	Flexner	Lee
Vice-President	Park	Ewing	Dunham	Dunham	Morgan	Morgan
Librarian	Lusk	Lusk	Lusk	——	——	——
Treasurer	Calkins	Calkins	Calkins	Calkins	Calkins	Lusk
Secretary	Gies	Gies	Gies	Gies	Gies	Gies

	1909–'10	1910–'10	1911–'12	1912–'13	1913–'14	1914–'15
President	Lee	Morgan	Morgan	Ewing	Ewing	Lusk
Vice-President	Gies	Gies	Levene	Levene	Field	Gies
Treasurer	Lusk	Lusk	Lusk	Norris	Norris	Murlin
Secretary	Opie	Opie	Wallace	Wallace	Jackson	Jackson

	1915–'16	1916–'17	1917–'18	1918–'19	1919–'20	1920–'21
President	Lusk	J. Loeb	Gies	Gies	Calkins	Calkins
Vice-President	Calkins	Gies	Auer	Auer	Wallace	Wallace
Sec'y-Treas.	Jackson	Jackson	Jackson	Jackson	Jackson	Jackson
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SAMUEL JAMES MELTZER, M.D.

Born, March 22, 1851—Died, November 7, 1920.

MEMORIAL NUMBER

FOR

SAMUEL JAMES MELTZER, M.D.

FOUNDER

AND

FIRST PRESIDENT

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

ADDRESSES GIVEN AT A MEETING OF THE SOCIETY HELD AT THE ACADEMY OF MEDICINE, JANUARY 6, 1921, IN ASSOCIATION WITH THE NEW YORK ACADEMY OF MEDICINE AND THE HARVEY SOCIETY OF NEW YORK

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1921

PRESS OF
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Minute expressive of the sentiment of the Society for Experimental Biology and Medicine on the death of Samuel James Meltzer.

The Society for Experimental Biology and Medicine has been deeply moved by the death of its revered founder, Samuel James Meltzer, who, from the beginning of its career, was the Society's devoted mentor and the personification of the Society's spirit and ideal.

Meltzer was eminent for many important contributions to biology, physiology, pathology, pharmacology, and scientific medicine. Most of his contributions to the advancement of science, after the Society's establishment in 1903, were made originally at meetings of this Society.

He was a distinguished promoter of the application of *experimental* methods to research in American medicine. His foundation of this Society was a particularly important means of accelerating that significant development in this country.

He was an enthusiastic embodiment of the spirit of zealous research, and an ardent exponent of idealism in science and in service.

He inspired fidelity to truth. He stimulated achievement in research. By example and precept, in the meetings of this Society for seventeen years, Meltzer appealed always to the best in every member. He quickened, in the oldest as well as in the youngest members, the impulses of emulation of his sterling qualities as a man, as an investigator, and as a servant of truth in every relation, that such attributes as his invariably elicit when radiated from an unselfish leader.

Meltzer's memory will be a continuing inspiration to the members of this society. Proceeding actively along the path his faithful leadership opened to us, and growing steadily in usefulness and strength, our Society will be not only an enduring monument but also a living testimonial to his achievements, his influence, and his character.

Deeply conscious of the personal loss that Meltzer's death involves for each of us, but earnestly grateful for the abiding value of his unbroken influence in our hearts, we dedicate ourselves anew to the promotion of the principles that Meltzer exemplified; and we are more firmly resolved than ever so to support and cherish this society that it may continue to be a worthy agency, of cumulative effectiveness—as Meltzer projected it—for the active advancement of science, for the exaltation of truth, and for the ennoblement of service, in biology and medicine.

Presented by the council and approved by the Society, at the one hundred and fourteenth meeting, held on March 16, 1921.

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Dr. Meltzer's relation to the Society for Experimental Biology and Medicine.

By **HOLMES C. JACKSON**, Secretary.

After twenty years of active investigation in experimental medicine and allied branches, it was quite natural that the scientific ideals of Dr. S. J. Meltzer should express themselves in a desire to form a society whose main purpose lay in stimulating experimental work among the younger men entering the fields of biology and medicine.

Accordingly, in conjunction with Dr. Graham Lusk, Dr. Meltzer sent an invitation to eight New York investigators to meet at Dr. Lusk's house on January 17, 1903, for the purpose of establishing a "Society for Experimental Biology and Medicine." This preliminary conference unanimously endorsed Dr. Meltzer's views and appointed a committee for permanent organization. The charter membership was increased to nineteen and the first meeting occurred on February 25, 1903, in the laboratory of physiological chemistry, College of Physicians and Surgeons, Columbia University. Dr. Meltzer became the first president of the new society and served two years.

Dr. Meltzer's first thought was to have the scientific program of the meetings presented in the form of demonstrations, and this idea was carried out during the first two years. As the society grew and the number of papers read at the meetings became greater, it was found necessary to alter somewhat this original intention so as to allow papers to be read by title.

During the first three years, the reports of the meetings of the Society appeared in *Science* and in *American Medicine*. In June, 1906, the decision was reached to publish the PROCEEDINGS as a separate journal, one number appearing after each meeting. The PROCEEDINGS has now established itself as a well-recognized and much sought for avenue of early publication for preliminary communications with an edition of 700 copies reaching all parts of the world. The meetings of the society have been held monthly each year from October to May inclusive, at the various educational institutions in New York City. In several instances out of town meetings in May were arranged at New Haven, Connecticut and at Cold Spring Harbor, New York.

Dr. Meltzer was a constant attendant at these meetings, adding immensely to their value by kindly discussion of the papers and by his clear cut and pointed criticism. His knowledge of literature was surprisingly accurate, diverse and extensive. He was a great reader and his retentive memory held all that came to his mind.

As a member of the council he gave much of his thought and energy to the various changes in policy which became necessary from time to time. To his mind the society functioned as a stimulus to scientific effort for the younger men in the various fields of biology and medicine. With this in view, he suggested the formation of branch societies in different parts of the country. Two of these branches, one on the Pacific coast and one in Minnesota, now meet every two months. Papers read at these meetings are published in the PROCEEDINGS. Dr. Meltzer lived to see the membership of the society become world wide and grow from fifty-six at the end of the first year, to four hundred and ten in 1920. Membership in the society is now recognized as a mark of scientific attainment; eligibility requires the publication of a "meritorious original investigation in biology and medicine by the experimental method."

Dr. Meltzer's death occurred on November 7, 1920. The funeral took place on November 10 from the Ethical Cultural Church, at which Dr. Simon Flexner and Dr. John Lovejoy Eliot delivered memorial addresses.

At the December meeting of the society it was voted to hold a memorial meeting at the Academy of Medicine in association with the Academy and the Harvey Society of New York. This meeting was largely attended; the president of the Society, Dr. Calkins, presided and addresses were given by Dr. George B. Wallace, Dr. Phoebus Levene, Dr. Graham Lusk, Dr. William H. Howell and Dr. William H. Welch. These addresses are printed in this memorial number of the PROCEEDINGS of the society.

Dr. Meltzer's example was a constant stimulus to the younger generation with which he came into contact, and the society feels deeply the loss which it has sustained by his death. The hand which guided the destiny of the society in the selection of its officers and members is no longer active. It is for others who remain to take up his task in holding fast the ideals which he impressed by his personality upon the society and its members.

Memorial remarks.

By **GARY N. CALKINS**, President.

Nearly eighteen years ago, or to be more precise, on the 17th of January, 1903, a small group of men, on the invitation of Dr. Meltzer, met to discuss the formation of a new society for the purpose of encouraging experimental work in the biological sciences. The following month the Society for Experimental Biology and Medicine was launched with 19 charter members, and Dr. Meltzer was its first president.

One of the most characteristic and lovable traits of Dr. Meltzer was his interest in young men and their progress in science. He saw in the National Academy a meeting place for those who had reached established heights in research and he liked to think of his new society as furnishing an opportunity for young men, fledglings in science, to try their wings. Always helpful to them with advice and by example, and always mindful of the highest ideals of scientific research he not only fathered the new society but he nursed it through its period of youth and watched its later growth with a jealous eye to see that the high standard of aims and ideals which he had set for it were maintained.

We meet tonight to do honor to his memory. The young society has grown and we believe, as we like to think, that his scientific spirit extends today throughout the entire membership of nearly four hundred active workers, and we like to think that the nickname which the Society early received—the Meltzer Verein—is synonymous with scientific idealism.

In recognition of this scientific spirit, and in respect to the memory of Dr. Meltzer, I am going to ask the members of the Society, and all others present who think as we do, to rise and remain standing for a few seconds.

A tribute to Dr. Meltzer's life and services.

By GEORGE B. WALLACE.

As the first speaker this evening and one who, through a friendship of some twenty years, has been largely influenced by Dr. Meltzer, I may be permitted to sketch in a somewhat general way those characteristics of his life and work which have especially impressed me.

My acquaintance with Dr. Meltzer began in the spring of 1902. At that time Professor Cushny was passing through New York and together we went, on what seemed to me a pilgrimage, to call on Dr. Meltzer at his house in Harlem. The visit stands out very clearly in my memory. Dr. Meltzer was then in his fifty-first year, in the full vigor of life and carrying on an extensive hospital and private practice. The conversation, however, was not concerning practice, but mainly on research work, his own and that of others. I recall how greatly I was impressed by his knowledge of the scientific work being carried on, by the clearness and fairness of his criticisms, and by his general enthusiasm and optimism. He dwelt at some length on the state of medical science in New York and deplored the isolation of the individual workers and their failure to meet at frequent intervals to present their work and exchange their ideas. Apparently this had been in his mind for some time, for he outlined a plan for the formation of a society which should include all the active workers in the biological and medical sciences. In the following year Dr. Meltzer put this plan into effect, and with the coöperation of a small group of his friends, the Society for Experimental Biology and Medicine was formally launched.

One of the last occasions on which I saw Dr. Meltzer was at a meeting of the Society held last spring in New Haven. He was then in his seventieth year and in miserable health, but his enthusiasm was as great as it had been twenty years earlier, and he had made what to him must have been a long and trying journey because of the intense interest he had in the society and in scientific work. As we walked very slowly and carefully back to his hotel, he told briefly and rather casually of the bad attack he had had the previous night, for which he had been obliged to call in a local physician, and then went on to describe the excellence of the meeting, the great field covered by the papers, and the fine spirit which was prompting the research work done in this country. This spirit, which he was so quick to see and appreciate in others, was, as a matter of fact, especially exemplified in his own life and work, and one feels in looking over his career, that it must have been the great driving force throughout his whole life, which made him indifferent to obstacles, difficulties and physical infirmities, great enough to daunt the ordinary man.

It can be truly said of Meltzer that he was a man who loved and pursued knowledge for its own sake. This was an inherent characteristic. When he entered the University of Berlin in 1876, it was the study of philosophy that attracted him particularly and it is probable that had his financial prospects been more favorable, he would have kept himself within this field, stimulated as he was by such eminent teachers as Paulson and Erdman, and by his quickly formed friendship with Steinthal. Moved by financial considerations, however, he entered at this time into the study of medicine. This was a day of great teachers and the University of Berlin was unusually fortunate in this respect. It is not difficult to see how the eager mind of young Meltzer must have been stimulated and indelibly impressed by contact with such masters as Du Bois-Reymond, Virchow, Leyden and Frerichs. It was at this time also that he began a friendship which influenced all his later life, namely, that with Kronecker. With his attractive, friendly personality, his thorough training in physiology under Helmholtz and Ludwig, and in medicine under Traube, his devotion to experimental science, Kronecker was the ideal guide and friend for the younger man just beginning a scientific career.

Shortly after his graduation, Meltzer came to New York and began the practice of medicine. He chose America, after careful deliberation, because its democratic form of government especially appealed to him. Previous to his settling in New York, he made several trans-Atlantic trips as a ship's surgeon, and his determination is shown by the fact that, in spite of his being a poor sailor and seasick during the greater part of each trip, he kept this position and carried on its duties for some time.

With the environment in which his medical education began, it is small wonder that Meltzer's interest turned to research work. What especially impressed him from the beginning was first, the necessity of careful observation and thoroughness in work, and, second, the importance of facts rather than theories. Those who have heard Meltzer present experimental work will, I think, recall numerous instances in which, when pressed for an explanation of his results, he has replied that although he had a theory, it was only the fact itself that he wished to bring out.

He began his research work in Kronecker's laboratory while yet a medical student. This work was on the swallowing mechanism and he himself was the subject of experimentation. He has given a graphic account of the discomforts endured during these experiments, for he was obliged to sit for hours with two stomach tubes, with rubber balloons attached to their ends, inserted into his esophagus. There is an interesting side-light connected with this work. While carrying on his experiment one day, the laboratory was unexpectedly visited by the Prussian Minister of Education, who inspected the laboratory and Meltzer in particular. The explanation of the visit came later. An anti-vivisection bill had been proposed, backed by the statement that experimentalists would not dream of inflicting on themselves the discomforts to which they were subjecting animals. The Minister was able to report that he himself had just witnessed a voluntary experiment on a human being which was attended by the greatest discomfort and he ventured the assertion that none of those who were so earnestly advocating the bill would be willing to put themselves in the place of the student in physiology. It may be added that the bill was defeated.

It was while engaged in this work that an idea was brought out, upon which most of his subsequent work centered. This was the phenomenon of inhibition. He had obtained a record of a single swallowing movement, but found that with successive repeated swallowings the record changed completely, in that the contractions failed to appear. In his perplexity he appealed to his friend Kronecker, who suggested the possibility of inhibition.

This possibility was eagerly seized upon and developed. It came in time to be the central idea upon which a large part of his research work was based. Briefly stated, his conception was that inhibition is as essential a process in cellular activity as is excitation. All living tissues are irritable, *i.e.*, they respond to stimulation with a vital reaction. This reaction can be either the manifestations of their specific activity, excitation, or it can be an inhibition of an existing activity. Absolute rest occurs when both opposing energies are exactly even and the difference between activity and rest consists only in the fact that excitation predominates during activity and inhibition during rest. To support his conception, Meltzer turned his attention to experimental proof. His studies on respiratory function strengthened his belief, as did his work on the gastro-intestinal tract. Later in his search for an agent causing inhibition, he discovered the depressing properties of magnesium and found in this substance what he believed to be the representative of inhibition in the animal body. His numerous papers on the action of magnesium salts are too well known to need review.

It may be noted here that owing to his wide knowledge gained through years of practice, he endeavored whenever possible to utilize his experimental facts for the service of humanity. Thus he pointed out and devised a technic for the use of magnesium sulphate as a general anesthetic and in the treatment of tetanus. He described its advantages in the treatment of burns and its application in the diagnosis of gall bladder disease. Suggested by the magnesium work, he turned his attention to artificial respiration and devised his method of intratracheal insufflation, a method notable for its simplicity, effectiveness and wide application.

In Meltzer's research work in general there is seen a breadth of view and range of subjects that is remarkable. In his earlier publications, as might be expected, there are found a number of papers on clinical subjects. Thus he wrote on the auscultatory sounds of swallowing, subphrenic abscess, congenital hypertrophic stenosis of the pylorus, otitis media and earache in pneumonia, paratyphoid, mechanical relations in the occurrence of pneumonia, myelopathic albuminosuria, gastralgia, intestinal colic and colic in general. These papers all show a keen observation and careful interpretation of facts.

From the outset, however, his interests were in purely scientific subjects. His first publication on the swallowing mechanism, with Kronecker, has been mentioned. Shortly after his arrival in this country he published with Professor Welch a paper on the behavior of the red blood corpuscles when shaken with indifferent substances. In this connection he was again fortunate, in forming an enduring friendship with Professor Welch, a friendship based on mutual respect and devotion to science. This paper was the forerunner of a large number on physiological subjects, too numerous to mention individually, which set a standard for American work and served as a stimulus for a great quantity of work by others.

There is another side of Meltzer's career which should receive special recognition. I refer to his part in shaping and hastening the development of scientific medical work in this country. Although the Society for Experimental Biology and Medicine, known affectionately as the "Meltzer Verein," held his particular interest, he was a leading figure in the organization of most of the present day American societies for medical research. His advice in matters of organization, policy, selection of members, was freely given and its value recognized. Of a highly altruistic spirit, his large experience and good judgment kept him from being impractical. His progressive point of view in all these matters is clear to one who reads his addresses, generally presidential ones. I quote as an example, extracts from his presidential address delivered before the Association of American Physicians. "The best physician of the future will be the man who has spent years in studying the methods employed in acquiring knowledge in the pure medical sciences and then in applying all his mental energies to a broad study of disease." "Clinical medicine and medical sciences must be brought closely together and work in harmony; that will assure a steady progress of the science and practice of medicine." "Some older members complain that the papers presented at the meetings are getting above their heads. While this may be a fact, it cannot be made the basis of a complaint. The papers of the program of our annual meeting reflect in general the character of the medical studies which prevail at that period."

Dr. Meltzer was especially instrumental in bringing about the formation of the American Society for Clinical Investigation, a society made up of younger men, active workers in medicine. This society is unique among medical organizations, in the character and ability of its members and the scientific excellence of their work. It is unquestionably one of the most important influences in this country in the progress of clinical medicine. His address at the first meeting of this society on "The Science of Clinical Medicine, what it ought to be and the men to uphold it" is an especially inspiring one, and sets forth fully the ideals for which he was fighting. Again a paper on "Headship and Organization of Clinical Departments" shows his conception of what the organization of a modern department of medicine should be. Through all these addresses one can find proof of his firm conviction of the successful future of medicine in this country. He had full faith in the new generation, with its education and scientific training.

I take the opportunity of quoting here a letter, recently received from Dr. Victor C. Vaughan, which reflects the general regard in which Dr. Meltzer was held by men of his own type. "I know of no one within my wide circle of acquaintances who has more fully filled my ideal of a physician and investigator than Dr. Meltzer. Although busy in the practice of medicine for many years, he always found time to do research, and this was of the highest kind. Personally I loved him like a brother. Professionally I appreciated his great service to science and to his profession."

And again, I quote from a letter received from Professor Yandell Henderson: "I believe that I can, as well as almost any one, testify from my own experience in scientific discussion with Dr. Meltzer, to the value of his constructive criticism. To excite comment on one's work from Dr. Meltzer was to receive at once stimulus, guidance, encouragement, and warning against premature conclusion. The function of critic which he filled for twenty years or more was one of his most valuable services."

In concluding, I feel that I can speak for the large group of men, young when Meltzer was in his full maturity, who looked on him as a sympathetic friend, a trusted adviser, an exponent of that spirit and accomplishment for which we are all striving.

Dr. Meltzer's message to the present generation.

By PHOEBUS A. LEVENE.

I am here tonight to speak not to the old friends of Dr. Meltzer who with him led the medical profession through the thorny walks of a primitive lowland to its present heights of splendor, I am here not to revive old memories so that some may again pass through the joys of their youth. I have come to speak to the younger, to deliver to them the message bequeathed by the older, Dr. S. J. Meltzer. And in order that they may receive the message I shall attempt to draw a sketch of the departed friend and master, not one portraying every detail of his character, not one bringing out every feature of his activities, but an impression portrait such as the young may hold before them while their life and ideals are still in their shaping. I shall throw the light on the side of the man that is the expression of the great ideal of service to humanity. The form of service is truly an incident.

It so happened that Dr. Meltzer was born in Russia, a country of irrepressible idealism; it so happened that he was born of a race noted for its devotion to whatever it chooses to make the object of its devotion; it so happened that he was born in a small modest town that gave birth to no bankers and to no magnates, but to many men of learning. Unlikely as this may seem, learning was the object of veneration in Dr. Meltzer's birthplace and learning became Dr. Meltzer's ideal.

In search of learning Dr. Meltzer migrated to Berlin, where he came under the influence of the great masters of medicine, of physiology, and of philosophy. In the atmosphere of these men his character matured, his ideals took concrete shape. Here the decision was formed that medicine was to become the medium of his service to man and here he chose physiology as the medium of self-perfection and of personal enjoinment. So earnestly did Dr. Meltzer apply himself to the task of mastering his medium that soon he gained not only appreciation but also the friendship and affection of those who had been his masters; and then, still early in his career, he was offered an opportunity of an academic position in Berlin. However, circumstances, among which not the least was an impelling desire for broader activity, induced him to decline the offer and again to migrate, and this time to our land.

With a background of Virchow, Helmholtz, DuBois Reymond, Koch, Frerichs, etc., Dr. Meltzer entered New York and on his arrival the contrast of past and present was not very cheering. Medical schools we had, but seats of learning they were not. Theory was not held in great repute, the largest space given to the laboratory was that occupied in announcements. On the school premises it was discovered with difficulty. The material the schools turned out was not of very high grade, but, such as it was, it formed the medical world which young Meltzer was about to join. Here to excel and to turn personal superiority into material gain was not difficult. Many men to whom Fortune was as friendly as to Dr. Meltzer, and who obtained the advantages of a European training, exploited their advantages successfully. Such success did not tempt Dr. Meltzer. On the contrary, from the day of his landing in New York his life was dedicated to the education and the advancement of the mental horizon of the American physician. There were other contemporaries who espoused the same cause, some were of American birth and had the advantages of a European education, and others of foreign birth and education. Prominent among them stand out Welch, Prudden, Janeway, Jacobi, Knopf in this city, again Welch and Osler in Baltimore, and others in other cities.

But among all these leaders who brought American medicine to its present high stand the place of Dr. Meltzer was from first to last unique.

To define his place among other leaders briefly, one would say it was more democratic. While others worked for the improvement of medical school or hospital, Dr. Meltzer chose for his task the education of the rank and file of M.D.'s, whether they were engaged in the practice of medicine or in the teaching of it. Again to borrow a term from the political vocabulary, Dr. Meltzer became the leader of the progressive opposition minority against the conservative majority. In order to exert his influence with the utmost efficiency, Dr. Meltzer chose to preserve his personal independence and because of this for many years he remained unaffiliated with school or hospital. Above that of personal independence, Meltzer held the necessity for leadership of one's continuous participation in active experimental investigation. Other leaders, whether educators or practitioners, early abandoned their habit of research. Over them Meltzer had an advantage. He also possessed the advantage of an indomitable craving for reading, and the advantage of a phenomenal memory.

Thus it happened that unaffiliated, holding no official position, Dr. Meltzer became the feared critic and the recognized leader and teacher both among the men of science and the men of practice. And often when new ideas and new discoveries in medicine had to be introduced to the American public, Dr. Meltzer was called upon to perform the task—and he always lived up to the occasion. His success in this direction lay in the fact that he never presented a subject before he assimilated it by experiment in the laboratory. Thus he labored and toiled to attain self-perfection and through self-perfection to aid and teach those around him.

Dr. Meltzer was one of the few men favored by Fortune who lived to see his efforts crowned with success. While his mental and physical energies were still in full vigor, the standard of the medical profession of America rose to unexpected heights. Dr. Meltzer could then devote more of his energies to his personal joys, things nearest to his heart—they were his old problems of physiology; old and many new. The opportunity presented itself with the foundation of the Rockefeller Institute. What he accomplished there, constitutes an important chapter of American medicine and more competent persons than I will give you an account of it. To me, however, tonight, his scientific contributions speak second and his life first. It was a simple life, simple in its dignity and honesty of purpose, magnificent in the humble manner of its great service to man and to ideal. The record of his life is the message Dr. Meltzer leaves not only to his colleagues, not only to the medical profession of America, but to all.

Personal reminiscences of Dr. Meltzer.

By **GRAHAM LUSK.**

A friendly personality so long a constant attendant at every important meeting of this Academy of Medicine has passed from us in the fulness of years and in the honored esteem of his fellowmen. Meltzer was born in Russia, educated in Königsberg, and then studied philosophy and medicine in Berlin between 1875 and 1882. In 1883 he came to New York and began the practice of medicine. He was deeply imbued with the scientific spirit of modern German medicine and was also a highly skilled practitioner.

At one of the clinics in Berlin, so he once told me, a patient was brought into the amphitheater and he, a student sitting in the top row of the circle of seats, was asked to make the diagnosis, to which he quickly replied that the trouble was cancer. "Nein," replied the professor. The question was put to several others who gave other interpretations and finally again to Meltzer who replied, "I told you, Herr Professor, the patient has cancer." A vigorous "Nein" was the rejoiner, the patient was passed from the room and the professor said, "Herr Meltzer, the patient has *carcinoma ventricularis*, but never allow yourself to tell anyone that he has a fatal disease." This, Meltzer said, he had carried with him as a lesson all his life.

Meltzer was not content to cultivate a lucrative practice at the expense of the extinction of his extraordinary, inquisitive mind. So one finds him taking holidays in the laboratories of his friends in Europe and, when at home in New York, he would go to the physiological laboratory of the P. and S., tie his horse to a lamp-post, and with his coachman as assistant, perform some physiological experiment for the comfort of his conscience and the instruction of his mind.

I well remember a dinner of the Association of American Physicians, held in the spring of 1897, an affair then always participated in by a few physiologists, at which I sat between

Meltzer and Chittenden, and my father sat opposite. After the dinner my father took my seat and explained to them that he considered the success of his book on obstetrics was due to the fact that he had begun his life as a trained physiologist. It was the kind of a beginning that appealed to Meltzer.

Meltzer was a great believer in associations of scientific men. In the many societies to which he belonged he was the most active member, continually discussing the work presented, and often pointing out similar work which had been accomplished twenty, thirty or fifty years before. His knowledge was phenomenal.

He became dissatisfied with the quality of the men in many of our scientific societies, criticizing them for their lack of activity, and out of this dissatisfaction sprang in 1903 the Society for Experimental Biology and Medicine, sometimes affectionately called the "Meltzer Verein," a name which, when he first heard, he indignantly opposed. Meltzer's original idea was that the society should consist entirely of *workers*, and that those who did not produce should be automatically dropped. But once the society was formed, the exigencies of warm personal friendships did not allow of the execution of the proposed penalty. However, it represented the central idea of his mind as to what a scientific worker should be.

At one of the meetings of the American Physiological Society, when it was suggested that the number of papers read by any one man be restricted, Meltzer opposed the resolution and offered a substitute to make it obligatory for every member to present a paper.

Once I spoke to him of retiring from active work at some indefinite future date, to which he replied, "No, you will never do it. You cannot. You will go on doing the little things you are able to do until the end, just as I shall. There are only two things which would stop me from working. If anyone said to me, 'Meltzer, your work is no longer good' then I would stop, or if anyone said to me 'Meltzer, you can no longer understand a young man' then I would stop also."

I remember that one evening Meltzer came to talk with me regarding the establishment of the Harvey Society which was designed to offer a forum for scientific speakers in New York. He opposed the idea at every point, saying that New York was not a scientific center and that there would be no audiences. Two or three days after that he telephoned me to call the meeting which had been proposed but I expostulated that he believed the affair doomed to failure. "Ah, but I have changed my mind," he replied. So the meeting was held with Meltzer in the chair and he overcame one after another all the arguments against the proposed society which a few days before he himself had felt as insuperable objections. Finally he said, "At any rate, we will all go and form a small group to encourage the speakers." At the first meeting Hans Meyer spoke and at the second this hall of the Academy was crowded to hear Carl von Noörden on the occasion of his first visit to America. And Meltzer's own lecture before the Harvey Society on the "Factors of Safety in Animal Structure and Animal Economy" attained world-wide celebrity.

A few years before this New York as a scientific center was pretty bleak and barren. In 1898–99 a few men, Lee, Herter, Dunham, Park, John Thatcher, Benjamin Moore, then in New Haven, and I, who were interested in laboratory work, met together informally at each other's houses and learned to know one another socially. This gathering was not resumed the following year. Then there sprang up a Society of Biological Chemists which met at regular intervals in the physiological laboratory of the New York University and Bellevue Hospital Medical College for the reason that this was the only institution to which access could then be obtained in the evening. This society afterward merged with the Society for Experimental Biology and Medicine and later gave its surplus of about a hundred dollars to help institute the Harvey Society.

It was in 1904, I think, after a meeting of the Society of Experimental Biology and Medicine that Meltzer drove me home from the P. and S. He took me across the park in his brougham drawn by a pair of horses. He said to me "I am going to give up all this. I am going to do what is nearest my heart. I am going to the newly founded Rockefeller Institute to spend the rest of my life in research. They allow me to practice medicine in so far as it pleases me, but my main desire is for experimental work."

This represented the spirit of his great love for scientific work. At one and the same time he not only fulfilled the desire of his life, but he renounced the material treasures of this world, and yet he remained free and untrammeled to do as he liked. On one occasion when I publicly mentioned this incident before him he regarded me with disapproval, and yet I believe it belongs to the story of his life. Very few men at the age of fifty-five would do likewise.

Meltzer was always a prominent figure at those international congresses which he attended. On such occasions the friendships between Meltzer and his old associate, Knonecker, were always warmly renewed. At the International Physiological Congress at Groningen in 1913 Meltzer, speaking in German, presented an eloquent and graciously worded invitation that the proposed congress of 1916 meet in New York. At the close of the speech a German turned to me and said, "Aber, Meltzer's Rede war schön!"

Like many of us who had known the better side of intellectual Germany, Meltzer was extremely cast down by the war. He sought to prepare the way for peace in his "Fraternitas medicorum" which was founded on the assumption that, since physicians of the Red Cross were bound to serve friend and foe alike, therefore, physicians themselves could readily resume friendly relations at the termination of hostilities. The supreme barbarity of modern warfare, however, has prevented the consummation of this altruistic hope.

Meltzer belonged truly to the younger men of his generation. For them he would make any sacrifice. He established the American Association of Clinical Research, the members of which were to be workers in the scientific sense. This society was so revolutionary that it earned the name of "The Young Turks." He was continually saying that in clinical medicine we had not yet reached a proper level of accomplishment, a level he hoped would still be attained in the future. It is usually hard for an older man to properly appraise those who are much younger than he. Liebig thought Voit a man without ideas, and Voit twenty years later knew of no prominent physiologist of forty years of age, at a time when Kossel and Hofmeister would both have been included in that category. However, Meltzer was not of the type to grow out of touch with the young men whom he had always so greatly encouraged and his judgment of them was not to be ignored.

He said to me one day, "Your ideas concerning medical education are certain to be accepted—not because *you* say them, but because they are right." These heartening words only illustrate the helpfulness of his spirit as vouchsafed to many. Honest words of strong condemnation or criticism from his lips also meant much to those of us who knew the texture of the mind behind them.

Last spring he said to me, "If my good friends at the Rockefeller Institute, out of affection for me and solicitude for my welfare, insist that I leave my laboratory there, I want to know if you will not permit me to work in your laboratory." He asked the same privilege of others. In the face of pain and suffering the indomitable spirit of the man could not be overcome.

We remember how, time and time again, Meltzer has sat among the front seats of this Academy next to his old friend, Abraham Jacobi, and we are grateful to have known one who has added by his own work and by his own personality so richly to the growth of New York as a center of medical science. The story of his life is of value to us all. Once he proudly remarked, "I am of the race of which came Jesus Christ." And, in fact, there are few men of our time who more completely embodied all the Christian virtues.

Dr. Meltzer's influence on American Physiology.

By **WILLIAM H. HOWELL.**

Dr. Samuel James Meltzer was born in Curland, northwestern Russia, March 22, 1851. He received his preliminary education in a Real Gymnasium in Königsberg and his later training in the University of Berlin where he graduated in medicine in 1882. After taking his medical degree he decided to make his career in America, as the country which in his opinion had the best form of government. He had not sufficient means to make the journey and was therefore obliged to secure a position as ship's surgeon on one of the transatlantic vessels. On arriving in New York it was necessary in the beginning to devote his time mainly to building up a practise sufficient to support his family, but almost from the beginning he made arrangements also to give part of his time to research. From that period until his death on November 7, 1920, in his seventieth year he was a tireless investigator. When in the course of time the opportunity came to him from the Rockefeller Institute to give his time entirely to research he did not hesitate in making his decision. At a considerable financial sacrifice he abandoned his medical practise to devote himself to the kind of work that he most loved and most valued. By his good work and his high character he attained a position of honor and distinction in American medicine and endeared himself to his fellow-workers in all parts of the country. His productivity was remarkable. The list of his published papers includes over two hundred and forty titles, distributed among some forty-eight scientific journals of this country, Germany and England. These papers contain contributions to the subjects of physiology, pharmacology, pathology and clinical medicine together with a number of lectures and general addresses. That he was an investigator of recognized standing in these several branches of medicine and was regarded as a valued contributor to so many scientific journals of the first rank is a striking demonstration of the breadth of his interests and knowledge. He was a member of twenty or more national scientific or clinical societies and in all of them it may be said he was prepared to take his part as an expert in the reading and the discussion of technical papers.

He served as president of the American Physiological Society, the Society for Experimental Biology and Medicine, the American Gastro-enterological Society, the American Society for the Advancement of Clinical Research, the Association of American Physicians and the American Association for Thoracic Surgery. The membership in these societies is composed of trained specialists. It is their custom to choose as their presiding officer only those who have made contributions of distinction to the subject to which the society is devoted. It seems to me unique in the modern history of medicine for one man to have received such special recognition from technical workers in so many different fields.

While his activities covered this large range he was interested primarily in physiology. "I belong," he said in a recent paper "to those who believe . . . that the knowledge of physiology is of special importance to clinical medicine." His work in this field entitles him certainly to be ranked among the foremost American physiologists. In attempting to present some estimate of the results of his labors I must limit myself mainly to his physiological activity. Indeed in this subject alone his papers are so varied that it will be possible to bring under review only what seem to be his major contributions. His first appearance as an investigator is recorded in a brief note in the *Proceedings* of the Berlin Physiological Society, May 14, 1880. In this note it is stated that Professor Kronecker exhibited a dog in which Herr Cand Med. Meltzer had cut the nerves going to the mylohyoid muscle and thus demonstrated the importance of this muscle in the initial stage of swallowing. At a later meeting of the society in the same year Kronecker presented the full results of an investigation carried out by Herr Cand. Med. Meltzer under his supervision on the "Process of Swallowing." This paper was published subsequently by Kronecker and Meltzer in the *Monatsbericht der Königl. Akademie der Wissenschaften zu Berlin*, 1881. In this important contribution the mechanism of swallowing was given an entirely new interpretation which has since been generally accepted and is known as the Kronecker-Meltzer theory of deglutition. Meltzer had attracted Kronecker's attention while a student in his course. Out of this acquaintanceship developed an invitation to engage in a research and eventually a warm friendship between the two men that lasted throughout life. Meltzer's career was thus determined while still a student of medicine. Kronecker's influence attracted him to physiology and set his feet in the paths of research. The investigation in which they collaborated was important and original—just what part each contributed it is not now possible to discover, but it is interesting to find that this initial venture into research furnished a motif which can be detected recurring again and again in Meltzer's subsequent work. A companion paper upon "Die Irradiationen des Schluckcentrums und ihre Bedeutung" was published by Meltzer alone in 1883. It is a very suggestive paper on account of the careful analysis it contains of the far-reaching and curious effects in the central nervous system of the act of swallowing and also because in it Meltzer announces certain views upon the importance of the inhibitory processes which subsequently formed the basis of his theory of inhibition, and remained with him throughout life as a sort of compass by which to set his course on his voyages of discovery. He calls attention in this work to the fact that reflex excitation of the inspiratory muscles is accompanied by reflex inhibition of the expiratory muscles and vice versa, and he goes on to make the suggestion that a similar relationship must prevail in the case of all antagonistic muscles such as the extensors and flexors of the limbs. Some ten years later Sherrington gave the necessary demonstration that this interrelation does hold with the muscular antagonists, that the contraction of the one is accompanied by the inhibition of the other and he designated this relationship under the term of "reciprocal innervation." Meltzer meanwhile had been accumulating instances of this combined action of excitation and inhibition, but he neglected at that period to apply a distinctive name to this kind of correlated activity. There can be no doubt that when it is possible to label an idea with an appropriate designation its currency in the scientific world is greatly facilitated. In his paper on "The Self-Regulation of Respiration" read before the American Physiological Society in 1889 and published in the *New York Medical Journal* and under a different title in the *Archiv. für Physiologie* he describes experiments intended to show that two kinds of afferent fibers exist in the vagus nerve, one exciting and the other inhibiting inspiratory movements. He used this fact to modify the Hering-Breuer theory of the self-regulation of the respirations by assuming that the expansion of the lungs stimulates both groups of fibers. The resultant effect, as in the case of the simultaneous stimulation of the motor and inhibitory fibers to the heart, is a dominance of the inhibitory effect, thus cutting short the inspiration and bringing on an expiration. But after the inhibition ceases the excitatory fibers, which, like the acceleratory fibers of the heart have a long after action, come into play and start a new inspiration. In his first general paper on inhibition this idea of a combined action of opposing processes is extended by the citation of numerous instances taken from physiological literature and is expanded into a general theory which makes inhibition a universal property of irritable tissues.

"I entertain and defend the view that the phenomena of life are not simply the outcome of the single factor of excitation, but they are the result of a compromise between two antagonistic factors, the fundamental forces of life, excitation and inhibition."

That is to say, whenever a tissue is stimulated two different processes are aroused, one leading to functional activity and one to a suppression of activity. As to the nature of these processes very little is said. He was not satisfied with the Hering-Gaskell conception that excitation follows or is an accompaniment of catabolic changes while inhibition is due to processes of an anabolic or assimilative character. He goes only so far as to assume that both processes are concerned with the kinetic and potential energies of the system, that excitation facilitates the conversion of potential to kinetic energy while inhibition hinders or retards this conversion, like the turning off or on of a stopcock. Nor was he satisfied with Sherrington's term of reciprocal innervation to describe all of the phenomena he had in mind. While this phrase is a suitable designation for the relationship between physically antagonistic muscles such as the flexors and extensors it is less appropriate in other cases, for example the simultaneous phases of contraction and inhibition exhibited in peristalsis. In later papers he suggested first the term *crossed innervation* borrowed from von Basch, but subsequently adopted the designation of *contrary innervation* as more applicable to the whole series of phenomena which he was considering. This process he believed is universal in its action—it is "manifest in all the functions of the animal body." Moreover his experience and observation as a practising physician led him to believe that "a disturbance of this law is a factor of more or less importance in the pathogenesis of many disorders and diseases of the animal body." In this way he would explain in part at least the muscular incoördination in tabes and the gastric crises of that disease, as well as gastric and intestinal colic in general. If the orderly sequence of a peristaltic wave is disturbed so that the advancing wave of contraction meets a contracted instead of an inhibited area conditions are present which may well bring about a distension sufficient to account for the pain of colic. He gives many other illustrations of pathological conditions which may find a plausible explanation on the assumption of a disorder or disharmony in the law of contrary innervation. How far Dr. Meltzer was correct in the applications of his theory it is not possible to say. In all probability some of the specific instances that he cites in support of his views are amenable now to other explanations. But it is a fact, I believe, that he was much in advance of his earlier contemporaries in the emphasis he placed upon the significance of inhibition in the general activities of the body. The story is far from being told but it may be said that physiological thought since 1883 has tended more and more toward some such general conception of the rôle of inhibition as was in Meltzer's mind. For him at least it was a rewarding theory, it played, as he expressed it, a dominating part in all of his researches. One can not wholly appreciate his work nor understand his position on controversial points unless this attitude is borne in mind. His theory of shock for example to which he held tenaciously was that "the various injuries which are capable of bringing on shock do so by favoring the development of the inhibitory side of all the functions of the body." There is a shifting of the normal balance toward the side of inhibition.

The most important of his contributions in later years will be found in three series of researches, one dealing with the action of adrenalin upon the blood-vessels and the pupillary muscles; one with the inhibitory action of magnesium sulphate and the antagonistic effect of the calcium salts, and one with the development of his method of artificial respiration by pharyngeal and intratracheal insufflation. The first series consists of eight or nine papers, mostly in collaboration with his daughter. They showed in this work that the temporary action of adrenalin upon the blood-vessels may be converted into a long-lasting effect, in the case of the ear-vessels, if these vessels are first denervated by section of the vaso-motor fibers in the sympathetic and the third cervical nerve. A more striking result still was obtained for the iris. In the mammal subcutaneous injections of adrenalin in moderate doses have no effect upon the size of the pupil, but if the superior cervical ganglion is first excised then, after a certain interval, subcutaneous injections bring on a marked and long-lasting dilatation. His explanation of these phenomena was made in terms of his theory of inhibition. Whether or not his views in regard to the relations of the cervical ganglion to pupillary dilatation will stand the test of future experimental work it is to be noted that the observation itself constitutes a significant instance of a kind of independent physiological activity on the part of a peripheral ganglion. The bearing of these facts upon the prevalent conception of the rapid destruction of epinephrin in the tissues was brought out especially in a paper with Auer in which it was shown that if adrenalin is injected into a ligated limb and an hour or so afterward the ligature is removed the dilatation of the pupil quickly follows, thus demonstrating that for this long period the adrenalin had remained unaffected by the tissues. Incidental results of this series of experiments were his discovery of the use of the frog's eye as a biological reagent for the detection of small concentrations of epinephrin and the rapidity of absorption in intramuscular as compared with subcutaneous injections.

The work upon the inhibitory and anesthetic effects of magnesium salts gave rise to no less than twenty five papers, most of them published in collaboration with one or another of his associates but chiefly with Dr. Auer. The peculiar inhibitory action of magnesium sulphate had attracted his attention as far back as 1899, and he reported upon it incidentally in a communication to the American Physiological Society. But in 1904–05, influenced again by his general conception of the importance of the inhibitory processes he took up with Auer a careful physiological study of its action. The results were most interesting and important. When given subcutaneously in certain doses the magnesium sulphate produces a condition of complete unconsciousness and muscular paralysis or relaxation, which is reversible, in the sense that when the animal is given proper care it recovers. Later he was able to show that out of this condition of profound depression or inhibition the animal may be restored to complete consciousness and motility with miraculous suddenness by the intravascular injection of small amounts of calcium chloride. No one who was fortunate enough to see this demonstration as given by Dr. Meltzer will forget its dramatic effect upon his audience. A healthy vigorous rabbit was brought quickly to a condition of complete immobility and apparent death by the magnesium sulphate and then even more suddenly raised from the dead and restored to its normal tranquil existence by the injection of some calcium chloride. Meltzer and his collaborators investigated various phases of this action of magnesium sulphate and all of the results obtained tended to strengthen in his mind the conviction that in magnesium he had discovered the element in the body that is especially concerned in the processes of inhibition. The antagonistic action of the calcium although exhibited in such a striking way was not in his opinion specific. His own experiments in connection with the results reported by other observers led him to the general view that calcium serves to balance the abnormal activity of the other kations, potassium, sodium and magnesium, whether this abnormal action is in the direction of excitation or of inhibition. Modern work upon the physiological significance of the inorganic constituents of the body fluids which was begun in Ludwig's laboratory, but was given its main impetus by the striking contributions of Ringer had concerned itself chiefly with the salts of potassium, sodium and calcium, which alone seemed to be sufficient to maintain normal conditions of irritability. Meltzer's work has shown that magnesium also has its place in this ancient balance of powers through which the functional activity of protoplasm is controlled. One can understand that in arriving at these results he must have felt that he was approximating at least a demonstration of the correctness of his general conception of the rôle of inhibition in functional activity. In this as in all of his experimental work Meltzer was eager to give his results a practical application to the art of medicine. The possibilities of the use of magnesium salts as an anesthetic agent in surgical operations were tested with some success on human beings and more important still its efficacy in controlling the spasms of tetanus has had a wide and promising application.

His last extensive series of researches dealt with anesthetization and artificial respiration through pharyngeal and intratracheal insufflation. Something like twenty-eight papers, most of them in collaboration with pupils or assistants, were devoted to this subject. His interest in this topic seems to have been stimulated by the fact that in his use of magnesium sulphate for anesthetic purposes the chief danger lay in the inhibition of the activity of the respiratory center. To meet this difficulty he undertook a study of the methods of artificial respiration. The initial paper in 1909 by Meltzer and Auer described a method of artificial respiration by continuous insufflation of the lungs through a tracheal catheter. It was found that by this means not only could an animal be kept alive without the action of the respiratory movements to fill and empty the lungs, but that it furnished also a convenient and efficient method for anesthetization. The use of this method in animal experimentation and especially its use in human surgery of the thorax and facial region was apparent and on many occasions Meltzer sought to make known its advantages and to ask for an adequate trial of its merits at the hands of the practical surgeons. The method has found some acceptance and the application of the principle involved will no doubt be extended in the future as the technique of thoracic surgery improves. It was in recognition of the importance of this work that the American Association for Thoracic Surgery asked him, a physician and laboratory worker, to serve as their first president. It was natural that this work should have led him to consider the whole matter of artificial respiration in its relations to resuscitation after accidents of various sorts. His general paper in the *Medical Record* for 1917 giving a history and critical analysis of the methods of resuscitation is an interesting and valuable contribution. He gives experimental data to prove that his device of intratracheal insufflation is the most efficient method of artificial respiration both for man and animals. But he realized that it is a method which requires special knowledge and training for its successful execution, and his broadening acquaintance with and interest in the practical aspects of resuscitation led him to experiment with the less efficient and less safe method of pharyngeal insufflation. He was a member of the three national commissions on resuscitation and served as chairman of the third commission. In connection with the duties of this service he devised a simple portable form of apparatus for pharyngeal insufflation which can be used with very little previous instruction and he demonstrated, with entire success I believe, that this form of apparatus is much more efficient than any of the so-called manual methods of resuscitation, or than any of the special machines for this purpose, pulmotors and lungmotors, which have been exploited commercially during the past few years. It was, I imagine, a sore disappointment to him that he was not able to convince his colleagues on the third commission that this apparatus met all the requirements for industrial and military use. It is probably the simplest and best instrument yet devised for artificial respiration as applied to man, and in institutions or industrial establishments where the need for artificial respiration may arise frequently and where special individuals may be instructed in its use it can be employed to great advantage. But it does require some little amount of training to use it properly—the average uninstructed man or woman can not be trusted to apply it intelligently, and for this reason the commission felt that it was wise to urge adoption of a manual method as the form of first aid which may be applied most successfully under ordinary conditions.

While the researches that I have attempted to summarize represent his most important contribution to physiological science, Dr. Meltzer kept in close touch with the progress in almost all branches of experimental medicine. He gave evidence of this interest in the publication of occasional papers on various topics or in articles of a general character. Shock, cardiac arrhythmias, therapeutics of self-repair, hemolysis, thyroid therapy, edema are among the subjects upon which he wrote, but probably the most original and helpful of his general papers is his well-known Harvey Lecture, 1906, on "The Factors of Safety in Animal Structure and Animal Economy." He applied this engineering term in a convincing way to describe the reserve powers possessed by many of the mechanisms of the body. Doubtless the general conception involved had occurred to many others, but no one before him so far as I know, had developed the idea so comprehensively and made of this provision a leading factor in the adaptation of the economy to its environment. The happy phrase that he employed served to precipitate the loose thought upon the subject, and its frequent recurrence since in medical literature is proof that the conception which it expresses has found wide acceptance in scientific circles. It is evident that his own thoughts were turned in this direction by the work of Chittenden upon the minimum protein diet. While he accepted, of course, the facts demonstrated by this observer in regard to the possibility of maintenance upon a low protein diet he was not willing to believe that a minimum diet is also an optimum diet in relation to the various metabolic stresses to which the body may be subjected. The experiences of the great war may serve to show that he was correct in taking this position.

To do full justice to the influence exerted upon contemporary medical research by Meltzer's work would require a careful analysis of the entire medical literature of the period, for, as I have tried to indicate, his sympathies were very broad and his activity was great. In some measure, either as interpreter or contributor this influence was felt at many of the points of contact between medical science and medical practise. The border land between these subjects was in fact his special field of work. He had the spirit and ideals of the scientist, and knew at first hand what research work really means. He had experienced the labor and care and devotion required of those who aspire to increase knowledge. On the other hand he had a personal realization of the difficulties and necessities of medical practise and so was especially fitted to act as a sort of liaison officer between the two great wings of the medical army, the investigators who have the difficult task of discovering new truths, and the practitioners who must learn to apply these truths to the preservation of health and the protection from disease. No one in our generation, I venture to say, was more useful in this country in bringing about a helpful and sympathetic understanding between the laboratory worker and the physician. As a physiologist he enjoyed the best opportunities and training of his period. He was equipped with the methods and technique that the subject owes to the great masters of the latter half of the nineteenth century. The more modern methods of physics and chemistry which seem to be essential for the new generation of physiological workers he did not possess, but he did not let this deficiency discourage him nor diminish in any way his activity in research. He had the wisdom to understand that the armamentarium with which he was provided was adequate for the accomplishment of much important and necessary investigation. He was no faint-hearted seeker after truth. There never was a time, I fancy, in his active life when his mind was not full of problems that he wished to solve and which he intended to solve in part at least with the aid of his experimental methods.

Dr. Meltzer was elected to membership in the American Physiological Society at its first annual meeting held in Philadelphia in December, 1888. From that time until his death he was perhaps its most faithful member in attendance, in the presentation of papers and in participation in the discussions and social intercourse. Other less heroic spirits might weary under the load of papers and seek respite and fresh air by frequent disappearances between acts, but this was never the case with Meltzer. He loved the meetings, he loved to listen to the papers and to take part in the discussions. He had something to say of value on almost every paper that was read. It is small wonder therefore that his position and influence in the society constantly increased in importance. He served as president from 1911 to 1913, but the older members know that before that time and since his advice was paramount in matters of policy as well as in the selection of officers. He was sincerely and deeply interested in the welfare of the society and believed in its importance as one of the major agencies concerned in the advancement of the cause of physiological research. What he had to say in regard to its policies was always said in the opening meetings and in the plainest of terms, and if in his opinion it was necessary to be critical of either persons or things he never hesitated to express what was in his mind. His courage in stating his position in matters in which some personal criticism necessarily played a part in the discussion has often aroused admiration. He did not indulge in circumlocutions or euphemisms, but was entirely frank and direct. There could be no mistake as to what he thought and yet no matter how plainly and bluntly he might speak there was as a rule no offense taken, because it was evident to every one that what concerned him was not personalities but the principles involved. The American Physiological Society owes much to him for the sound policies and wholesome traditions which have characterized its history. I have not so much direct knowledge of the influence exerted by Dr. Meltzer in the numerous other societies of which he was a member. In the case of the Society for Experimental Biology and Medicine we know that he was its chief founder and for many years its primum movens—it was long known familiarly among scientific men as the Meltzer Verein. I have no doubt that in every organization with which he was connected his influence was always exerted on the side of the highest scientific ideals—no other position was possible for him. He was high-minded, courageous, sincere and optimistic. Age oftentimes lays a stiffening hand upon the scientific worker, causing him to shrink from the laborious routine of research, but with Meltzer there was never any indication of weariness or sense of failure. In spite of much ill-health and physical suffering in his later years he was full of hope and energy and determination in the pursuit of his scientific ideals and problems. Death came to him, as he would have chosen, while in his study and at his work. He was a good and faithful servant in the cause of medical research. Rewards came to him in the form of academic honors and membership in the most important medical and scientific societies, but I am confident that he found his greatest recompense in the joy of the work and in the affectionate appreciation of his many scientific friends.

The place of Dr. Meltzer in American medicine.

By WILLIAM H. WELCH.

It seems as though every side of Dr. Meltzer's life and work has been already touched upon. There is little I can add. At the same time I should regret very much not to have had the opportunity of coming here tonight and paying my tribute to the memory of one whom I have held dear ever since the beginning of our acquaintance, which dates from Dr. Meltzer's arrival in this country in 1883. The bond that brought us together was one already refered to; we were both pupils of Kronecker, I at an earlier time than Meltzer, when Kronecker was assistant to Ludwig in Leipzig. I had the good fortune of coming under the influence of Kronecker and enjoying a friendship which continued until his death. Everyone who had the opportunity of working with him, loved him. He took a special and permanent interest in all of his pupils.

When Meltzer came, or even before he came, I received a letter from Kronecker informing me that Meltzer was contemplating coming to this country, and inquiring whether it would be possible for him to secure some academic position. If Dr. Meltzer desired to secure an academic position when he came here, he was soon disillusioned when he saw what the conditions at that time really were.

Occasionally Meltzer would pour out his heart, and I have had a letter from him within the year, a very intimate one, giving the circumstances of his drawing out from his early environment. He describes in a very graphic way the small city in Russia where he lived with his family circle, a circle of very orthodox Jews with a remarkable love of learning but at the same time a very restricted horizon. There is no little pathos, and some romance which I hardly feel free to tell here, of the circumstances which led him, under the particular influence of one individual, to leave his home and go to Koenigsberg there to enter into another life, another spirit, another world of thought.

He came to this country in 1883 with an admirable training in medicine but with his interests centered in experimental physiology and particularly in that field of experimental physiology represented by Kronecker. Although a pupil in the DuBois laboratory, it can hardly be said that the character of DuBois' work was the one in which Meltzer was trained or which especially attracted him. He arrived here with a letter from Kronecker and appeared in the little laboratory where I had been for four or five years after my return from Germany—first one room, then finally three rooms in the old Bellevue Hospital Medical College. I was delighted to have him. I recall that about that time Dr. Lange, the surgeon, was working in the laboratory before he had established himself in practice, and I could not give Meltzer a separate room. He had merely a corner in the laboratory and he was a faithful attendant there. He came every day as I recall it, usually in the afternoons, and there we undertook and published together a little piece of research, Meltzer's independent work practically. I never quite followed him in some of the broader views he subsequently elaborated and based upon that work as to the importance of vibratory movements in living matter. In that apparently detached kind of study he had a breadth of view somewhat philosophically tempered.

That association which was a great delight to me lasted one year. He then moved to Dr. Prudden's laboratory. Dr. Prudden and I had started our laboratories at about the same time. Those laboratories were then practically the only ones in New York where anyone who desired to do any kind of biological or medical laboratory work, could come. There can be no greater contrast than the conditions in those very modest little laboratories and the splendid equipment of today. This is possibly a good illustration of the "lowlands" of those days as compared to the "heights" of today, and no small influence in bringing this about was that of Meltzer's. But although working for a time in the laboratory of the College of Physicians and Surgeons, most of his research was done in his own little house, often late at night.

I would like to emphasize what I think is the marvel of Meltzer's life and work, that remarkable and almost unique combination of active medical practice with the cultivation of a particular science, experimental physiology by laboratory methods. There have been physicians, especially in their younger and lean years, with scientific inclinations who have done excellent work in the laboratory but only for a time; ultimately they were practitioners. There have been practitioners, as for example S. Weir Mitchell, who have continued to be interested in experimental work, but after all they are not comparable to Dr. Meltzer, who combined in an extraordinary manner the life of the practitioner and the life of the real specialist in experimental physiology. It is worth pausing to consider this because it is a remarkable phenemenon. Meltzer must have exerted no little restraint not to allow himself to become so immersed in practice as to cause him to withdraw from his scientific activities.

The first ten years after his arrival were years in which he produced something nearly every year. There were some years, 1884–85–86, of relatively little productivity. In general the period from 1883 to the early nineties of the last century was one in which he was establishing himself in a comfortable practice; he desired no more. He had to make his livelihood and this was the only congenial way open to him, but he did not allow himself, even during these early years, to be withdrawn from scientific interests and work. It shows a remarkable loyalty to an ideal and a very extraordinary enthusiasm and tenacity of purpose to have accomplished this under conditions apparently so adverse. Once established in a comfortable practice, his scientific interests bore upon his practice and his practice bore upon the character of his scientific progress, as has been already pointed out.

Dr. Howell has given us an admirable characterization of Meltzer as the experimental physiologist who occupies by preference that border land between laboratory and practice, a type quite incomprehensible to the ordinary practitioner.

Meltzer was one of the few earlier physicians in this country whose practice was based upon physiological training, aptitude and interest. S. Weir Mitchell was another, although his interests became mainly clinical. If Dr. Graham Lusk had not already referred to it, I was going to speak of his father also, as another man who, in his special field of obstetrics, founded upon physiological study and interest, made admirable the work of the scientific practitioner in this field.

In those early years when Meltzer came to New York, the leading physicians were Jacobi, Clark, the elder Flint, Delafield and Janeway, the scientific basis of whose work was mainly pathological anatomy. From this school of pathological anatomists most admirable practitioners have come; but today we recognize that the study of function is essential to make the good doctor, and we must bear in mind that Meltzer typified this idea when scarcely any one else in the country did so.

In the early nineties Meltzer's productivity amounted to many papers a year, and so continued to the end. It is interesting to consider why. In the first place he was in easier circumstances, not uninterested in his practice, but easier in his circumstances so far as time to give to his work went. Then it was a time too, when there were great advances in scientific and medical education. Laboratories were established in various schools in the country. I wish to emphasize also the formation of special societies such as the Association of American Physicians, devoted to the various specialties, particularly the Physiological Society, where Meltzer played the very important part indicated by Dr. Howell. At this time too, there came the establishment of journals devoted solely to the publication of technical research. They not only provided a much needed means for publication, but they were positively stimulating to the production of research. The first of these was the *Journal of Experimental Medicine* started in 1896, soon followed by the *Journal of Physiology* and then by others. The organization of these special societies, the new media of publications, gave Meltzer his opportunity, and how well he used it, how much a part of this development he was, has been indicated here tonight.

And now, just a little more about his relation to the clinical side. He represented the physiological type of physician. He was, I understand from competent sources, really an accomplished physician, doing full justice to his patients. His influence on clinical medicine however, is not to be measured by his accomplishments merely as a physician. He was never weary of impressing especially upon the younger generation of physicians, that the field of clinical research is just as interesting, as rewarding, as important and just as capable of scientific advancement by research as that of physiology or the other branches of medicine to which the term "science" is sometimes, although erroneously, limited.

Meltzer realized that it is the younger generation that is especially worth working for and trying to influence. He made no mistake. It is delightful to see so many of the younger men here tonight, because I know they are drawn by their affection and admiration for that man who impressed his ideas upon them not only by precept, but by example. In this way, I think, he has exerted a potent influence upon clinical medicine, and I question whether it would have been possible for a man devoting himself solely to laboratory work to have done this.

His great opportunity came when he was chosen for the headship of the division of Physiology and Pharmacology of the Rockefeller Institute. It was a natural choice. The institute was in its early days and it was extremely important that the particular problems selected for study should fall within a certain, at that time, well-defined group of subjects. Here was a man who was a genuine physiologist, recognized by his compeers, but whose interests were largely concerned with problems having relation to practical medicine, although he would have been the last man in the world to advocate that practical application of results should be a guiding principle in discovery. He represented that combination of quality and direction of interest in scientific medicine which made him the ideal man for the new division of physiology and pharmacology. He found here his great opportunity for his splendid work. He was brought into contact with young men and he helped train them here.

As a member of the Board of Scientific Directors of the Rockefeller Institute, I wish on this occation, to express on behalf of all my colleagues, and I am sure I speak also for all the scientific workers at the Institute, our sense of personal loss, our very grateful appreciation of Meltzer's life and work and our inexpressible debt to him for his many years of devoted and fruitful service. I am glad to have had the privilege of saying these few words in memory of one whose character I greatly admired and whose friendship I cherished. It is well for all of us to come and pay our tribute to the memory of such a man; to recall his worthy qualities of heart, mind and character, his large and enduring influence, his accomplishments and his genius. But after all, it is still better for us, and that is what he would wish, that we leave here animated by his spirit and by his desire to cultivate scientific medicine and to serve our fellow men.

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED NINTH MEETING

CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK CITY
OCTOBER 20, 1920
AND
TWENTY-SIXTH MEETING
PACIFIC COAST BRANCH
BERKELEY, CALIFORNIA
OCTOBER 13, 1920

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NEW YORK

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EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED TENTH MEETING

NEW YORK POST-GRADUATE
MEDICAL SCHOOL
NEW YORK CITY
NOVEMBER 17, 1920

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ONE HUNDRED ELEVENTH MEETING

ROCKEFELLER INSTITUTE
FOR
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NEW YORK CITY
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Bailey, Baehr, Calkins, Chambers, Coleman, Eddy, Funk, Gies, Greenwald, Jackson, H. C., Jobling, Kleiner, Little, MacNeal, Maltaner, McCann, Mueller, Myers, Olitsky, Papenheimer, Rose, Sherman, Sherwin, Sittenfield, Stark, Thro, Waksman, Wallace.

Members elected at the hundred and tenth meeting:

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Date of the next meetings:

December 15, 1920.

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Fordham University School of Medicine.—A. O. Shaklee, Carl P. Sherwin.

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Winnipeg, Canada, Dominion Grain Laboratory.—F. J. Birchard.
Germany.—Reinhard Beutner.

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Auer, Burton-Opitz, Calkins, Churchman, DuBois, Edwards, D. J., Goldschmidt, Hess, Jackson, H. C., Lusk, Moore, Myers, Olitsky, Pappenheimer, Rose, A. R., Torrey, Uhlenhuth, Waksman.

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Dates of the next two meetings:

November 17, 1920–December 15, 1920

PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED TWELFTH MEETING
COLLEGE OF PHYSICIANS AND SURGEONS
NEW YORK CITY
JANUARY 19, 1921

AND

TWENTY-SEVENTH MEETING
PACIFIC COAST BRANCH
BERKELEY, CALIFORNIA
JANUARY 12, 1921

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SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED THIRTEENTH MEETING
COLLEGE OF THE CITY OF NEW YORK
NEW YORK CITY
FEBRUARY 16, 1921

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AND
TWENTY-EIGHTH MEETING
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ONE HUNDRED FIFTEENTH MEETING

UNIVERSITY AND BELLEVUE HOSPITAL
MEDICAL COLLEGE

NEW YORK CITY

APRIL 20, 1921

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SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED SIXTEENTH MEETING

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NEW YORK CITY
MAY 20, 1921
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Members elected at the one hundred fifteenth meeting:

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Dates of the next two meetings:

May 18, 1921–October 19, 1921.

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